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The Journal of Laboratory and Clinical Medicine

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CLINICAL AND EXPERIMENTAL

FURTHER OBSERVATIONS ON PATIENTS WITH HIGH BLOOD SUGAR BUT NO GLYCOSURIA*

RALPH H. MAJOR, KANSAS CITY, KAN.

IN 1925 Major and Davis[1] described eight patients suffering from diabetes mellitus who were under treatment with insulin and showed a high blood sugar with no sugar in the urine. The ages of these patients varied from eleven years to seventy-two years and height of the blood sugar elevations from 196 mg. per 100 c.c. to 425 mg.

FOLIN-WU METHOD

NO.	AGE	BEFORE FERMENTATION	AFTER FERMENTATION	URINE
1	26	500	71	Neg.
		434	36	Neg.
		444	19	Neg.
2	63	189	36	Neg.
3	35	200	18	Neg.
4	51	230	28	Neg.
		218	30	Neg.
5	56	247	30	Neg.
		200	24	Neg.
6	65	333	32	Neg.
7	58	227	33	Neg.
8	20	363	28	Neg.
9	17	250	34	Neg.
		307	36	Neg.
		238	18	Neg.
10		267	28	Neg.
11	20	244	20	Neg.
12	59	303	42	Neg.
		500	41	Neg.
		236	28	Neg.
13		207	32	Neg.

*From the Department of Internal Medicine, University of Kansas School of Medicine.
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The observations of Somogyi[2] showing that a part of the substance in the blood which reduces copper sulphate is not glucose since it does not ferment, suggested a further study of patients showing high blood sugars but no sugar in the urine. It seemed theoretically possible that the apparent high blood sugars might be due to the presence in the blood of substances other than sugar, which could not be excreted in the urine. This question has been studied further in sixteen patients who showed high blood sugar but no sugar in the urine. In these patients the blood and urine specimens were collected simultaneously.

The blood filtrates were prepared according to the method of Somogyi. Sugar determinations were carried out in one series of observations by the Folin-Wu method and in the second series by the Shaffer-Hartmann method.

SHAFFER-HARTMANN METHOD

NO.	AGE	BEFORE FERMENTATION	AFTER FERMENTATION	URINE
1	45	241	39	Neg.
		274	34	Neg.
		237	44	Neg.
2	59	172	34	Neg.
3	30	201	41	Neg.
4	49	183	31	Neg.
5	48	183	39	Neg.
6	51	359	34	Neg.
		203	23	Neg.
7	63	279	29	Neg.
		333	31	Neg.
8	66	200	29	Neg.
9	55	196	18	Neg.
10		196	18	Neg.
11	51	194	29	Neg.
12	77	176	36	Neg.
13		260	34	Neg.
14	10	203	31	Neg.
15	64	225	39	Neg.
16	20	241	29	Neg.

These observations show that the high values for the blood sugar in these patients are due to a fermentable sugar, presumably glucose, and not to any other nonexcretable substance. The absence of sugar in the urine is evidently due to what is commonly termed a high renal threshold for sugar.

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THE ANTISEPTIC AND BACTERICIDAL ACTION OF UREA*

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UREA, historically significant as the foundation stone of modern synthetic organic chemistry, is generally regarded as nothing more than a convenient and, perhaps, from the standpoint of energy, an economic form in which the mammalian organism can excrete nitrogen. Yet a close search of the literature reveals isolated reports of biologic activities of urea which may be of considerable practical value.

In an account of the action of urea upon proteins, Ramsden[1] made the very interesting observation that urea prevents putrefaction. Such an action, it appeared to us, could be due either to the suppression of enzyme activity or, more important still, to the inhibition of growth of those organisms which fabricate proteolytic enzymes. Experiments by one of us (J. H. F.) show definitely that enzyme action may be retarded by low concentrations of urea. The present paper reports results obtained by the use of saturated solutions of urea in the treatment of purulent exudates and data on the bactericidal action of urea (by L. F.).

The first detailed study of urea as a bactericide is that of Péju and Rajat.[2] Following the observations of other workers, whom they do not specifically mention, these authors studied the polymorphism produced in bacteria by urea. They found that on media containing urea there was less and less bacterial growth until media saturated with the compound remained sterile. Coincident with the decrease in growth there was, in some cases, an increase in the number of abnormal morphologic forms. There were present sometimes long, empty, unbranched filaments of uniform, homogeneous structure and sometimes large oval or pyriform masses or filaments with such masses at the extremities or occasionally on their length. Ultimately, and after a period characteristic for each organism, the bacteria, if left in the urea medium, tended to revert spontaneously to their normal forms. According to the ease with which they were affected by urea, the organisms studied were divided into three classes:

I	II	III
B. Eberth	*B. cholerae* (Calcutta)	*B. violaceus*
B. diarrhea (green)	*B. Tholler*	*B. septicus aerobicus*
B. psittacosis (Nocard)	*B. butyicus* (Binot)	*B. mycoideus rosaceus*
B. pyocyaneus	*B. Korn 1*	*B. anthracis*
B. dysentery (various types)	*B. Gross*	*B. diphtheriae*
B. enteritidis (Gartner)	Mistbacillus	Various cocci (staph., strep., pneumo., sarcinae)
	B. tuberculosis (human)	

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The bacteria in Group I were easily modified by urea, polymorphism being produced readily and rapidly. Those in Group II were not easily modified, often requiring several weeks for a slight change. The organisms in Group III showed no polymorphism in presence of urea. No information was given which would allow of correlation of polymorphism and growth in media of different urea strengths.

Wilson[3] found that 8 per cent urea prevented the growth of *B. coli*, while 1.5 to 3.5 per cent caused polymorphism.

No great attention was paid to the bactericidal action of urea until Symmers and Kirk[4] reported bacteriologic and clinical studies. They found a number of interesting and important facts, which can be summarized as follows:

a. Urea prevents bacteria from deoxidizing blood.

b. No growth could be obtained on tubes inoculated from twenty-four-hour agar culture of *B. typhosus* which had been subjected for thirty minutes to the action of a saturated solution of urea.

c. An old, putrid, tuberculous sputum, which gave, on agar, confluent growths of various organisms, was treated at room temperature with a saturated solution of urea. After fifteen minutes a loopful was smeared on agar. Only three colonies grew. After thirty minutes' action of urea only one colony grew.

d. Five cubic centimeters of blood were inoculated with *B. pyocyaneus*. Controls gave abundant growth, but transplants from the mixture after it had been treated with 2.5 gm. of urea for thirty minutes at room temperature were sterile.

e. A similar mixture containing 1.25 gm. of urea gave ninety-eight colonies on an agar transplant after standing fifteen minutes, while after standing thirty minutes only five colonies grew.

f. Below a concentration of 25 gm. of urea per 100 c.c. of blood there was no definite bactericidal action, but growth was delayed.

Symmers and Kirk then proceeded to clinical tests of the bactericidal action of urea. At first they used a fresh 100 per cent aqueous solution. This they found of undoubted value as a wash in the treatment of diphtheria carriers. In the treatment of wounds closer and more continuous contact with urea was needed. Therefore the infected tissues were liberally dusted with the powdered compound and the wounds finally closed with continuous sutures to prevent drainage. That urea is innocuous to human tissues was adequately proved. Thiersch epithelial skin grafts over healthy ulcers never necrosed in presence of urea. A simple, oblique fracture of the shaft of the femur, in a young man, was treated with 6 gm. of powdered urea, which was placed alongside the fracture after plating. The muscles, fascia lata, fat and skin layers were closed separately with continuous sutures. There was no sign of interference with healing, and in three weeks all splints and dressings were discarded and massage and passive movements commenced. In many radical operations for inguinal hernia, urea was placed between the different tissue layers without damage. In one case with a chronic staphylococcus blood infection, urea was sprinkled between the layers of tissue and the wound then closed with continuous sutures. Healing followed with no sign of infection. Sloughing, infected wounds, dressed with urea powder once in twenty-four hours, gave better results than similar wounds treated by other methods. The absence of venous congestion in tissues was

noticeable in contrast with cases treated with ordinary wet dressings, continuous irrigation, or frequent baths. The circulation was normal and repair seemed more rapid. There was no irritation.

The only disadvantage noted with the urea treatment was occasional severe pain, which, however, could be controlled by morphine. Since urea absorbs moisture rapidly, the powdered solid easily became caked. This caking was prevented by covering the urea layer with oiled silk and the latter with a dry dressing. The wounds required dressing only once in forty-eight hours.

Unaware of the work of Symmers and Kirk, one of us (J. F.) selected as material for a clinical study of urea a few cases of purulent otitis media, complicating contagious diseases in children. All the patients studied were on the Pediatric Service of Cincinnati General Hospital. In most cases the primary disease was scarlet fever and the ears were infected with a hemolytic streptococcus. Also, most of the cases had failed to respond to treatment with mercurochrome, hydrogen peroxide, silver preparations, etc. The technic used was simple. A fresh, saturated aqueous solution of urea was instilled into the affected ear every four hours, using a medicine dropper. Since it was soon found that the urea crystallized out and plugged the external canal, thus hindering drainage and preventing observation, the ear, before each instillation of urea, was gently irrigated with normal salt solution, again using a medicine dropper.

The number of cases treated (20) is too small for statistical study. The results of the treatment were in general as follows: All of the cases which had failed to respond to other local medicaments responded to urea. The discharge, often of a foul odor, lost its foulness in a few hours and became serous or mucoid. In from three to six days all discharges ceased. Smears from ears which originally contained hemolytic streptococci no longer showed this organism, but often a hemolytic staphylococcus not previously present was found. There were no signs of irritation.

Cases treated with urea at first onset of otitis media almost all responded rapidly, sometimes clearing up after thirty-six hours and usually within three to six days. Twice discharges were checked but recurred a week or two later, after urea treatment was stopped. In both cases a mastoiditis was present and was later treated by radical operation.

Two results of urea treatment are of special interest. The first patient, a boy of ten years, developed a bilateral otitis media and a hemorrhagic nephritis about the third week of hospitalization with scarlet fever. The ear condition failed to respond to mercurochrome, hydrogen peroxide, and silver preparations. Macroscopic and occult blood were present in the urine, which was examined daily. After three weeks of this condition urea treatments were started. The ear discharges at once became less foul and more serous. At the same time the blood gradually disappeared from the urine. After ten days of urea treatment only a slight serous discharge was present and tests for blood in the urine were negative. The discharge never entirely ceased until mastoid operations were performed.

The second patient, a girl of five years, was admitted with a severe streptococcic throat infection. The nose and submaxillary lymph nodes were soon involved. The latter were opened and drains inserted. In spite of persistent

treatment with potassium permanganate, hydrogen peroxide, and other preparations, the infection spread. The patient became delirious and finally comatose. The foul odor of discharges from the nose, mouth, and lymph nodes permeated the ward. When no other treatment availed and the child was moribund it was decided to use urea. The nose and throat were irrigated with a saturated solution of urea every two hours. The draining lymph nodes were packed with gauze soaked in a saturated solution of urea. Within twenty-four hours the odor of the discharge was diminished and had disappeared in three days. The patient died a week later from peritonitis.

One of us (L. F.), interested in these clinical results, has again tested the bactericidal action of urea.

Fresh twenty-four-hour cultures of thirteen common species were washed from appropriate mediums in exactly 5.0 c.c. of sterile saline and tubed. The turbidity of each suspension was measured against the usual fuller's earth standard. Then to each tube was added exactly 5.0 c.c. of a freshly prepared saturated solution of urea in distilled water and the suspension thoroughly mixed. The tubes stood at room temperature throughout the entire period. After two hours of exposure to half-saturated urea, 1.0 c.c. was withdrawn from each tube and transferred to other tubes containing 15 c.c. of sterile saline. All tubes were centrifuged until the supernatants were clear. The bacteria were washed once in saline, and the entire final sediments were transferred to appropriate mediums to determine viability. This procedure was repeated at three hours and again at four hours of exposure. After twenty-four hours the original urea-bacteria suspensions were washed twice with saline and large loopfuls of the final heavy sediments were similarly transferred to appropriate mediums. Meat extract broth was used for the hardier species; dextrose ascites broth for the streptococci and staphylococci; and dextrose cystine ascites hemoglobin agar for *B. tularense*. All culture tubes were incubated at 37° C. for five days before discarding. The viabilities of these species after exposure to half-saturated urea are shown in Table I.

TABLE I

VIABILITY AS DETERMINED BY CULTURE

SPECIES USED	TURBIDITY OF ORIGINAL SUSP.	EXPOSURE TO HALF-SATURATED UREA 2 HR.	3 HR.	4 HR.	24 HR.
B. coli communis	3000	0	0	0	0
(2 strains)	3000	0	0	0	0
B. coli communior	3000	0	0	0	0
(2 strains)	3000	0	0	0	0
Staph. aureus	2000	+	+	+	+
Staph. albus	2000	+	+	+	0
Strep. fecalis	800	0	0	0	+
(2 strains)	800	0	0	0	0
Strep. viridans	100	0	0	0	0
B. mucosus	2500	0	0	0	0
(2 strains)	2000	0	0	0	0
Prot. vulgaris	2000	0	0	0	0
B. typhosus	2000	0	0	0	0
B. Para-Ty. B.	2000	0	0	0	0
B. aerogenes	1000				0
B. fecal. alk.	1000				0
B. tularense	1000				0

It will be noted that the staphylococci resist the action of urea better than other species tested, especially *Staphylococcus aureus*, which survived after twenty-four hours of exposure. *Staphylococcus albus* survived for four hours of exposure, but not after twenty-four hours. One strain of *Streptococcus fecalis* was viable after twenty-four hours, but growth was not obtained at the earlier periods. The last species were tested only after twenty-four hours of exposure.

The results so far obtained suggest that urea may be of considerable value in the treatment of purulent discharges of many types and in the treatment, also, of suppurating wounds producing foul odors. This latter use of urea has been reported recently by Millar.[5] His paper has received rather severe criticism only because it has been generally believed that urea has no bactericidal power. The cheapness and harmlessness of urea should encourage other investigations of its clinical use.

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PERIPHERAL NEURITIS AND ABORTION FOLLOWING DINITROPHENOL THERAPY*

Report of a Case

Ervin Epstein, M.D., and Harold Rosenblum, M.D., San Francisco, Calif.

ALPHA-DINITROPHENOL ($C_6H_3(NO_2)_2OH$) was introduced into medical therapy by Cutting, Mehrtens, and Tainter[1] for the treatment of obesity. This compound was of clinical importance during the World War, however, since numerous instances of dinitrophenol poisoning were reported among munition factory workers at this time. The purpose of this report is to present an instance of peripheral neuritis and abortion occurring in an obese woman following the administration of dinitrophenol.

REPORT OF CASE

Mrs. A. S. is a white, married, housewife aged thirty-four years. She is the mother of three normal children and had had no abortions or miscarriages. The remainder of her past history and family history were irrelevant.

The patient was treated in the Metabolic Clinic of the Mount Zion Hospital for obesity. On Feb. 20, 1934, treatment for this condition was instituted with an initial daily dose of 100 mg. of sodium dinitrophenol. This is the equivalent of approximately 75 mg. of the acid 1-2-4 dinitrophenol. The daily dose was increased to 200 mg. of sodium dinitrophenol daily on March 6, 1934, and further increased to 300 mg. one week later. There was no further change in the dosage until April 3, 1934, when it was increased to 400 mg. and then to 500 mg. one week later. On this therapy she lost 21.5 kilos (47¼ pounds) from 107.0 kilos (235½ pounds) to 85.5 kilos (188¼ pounds) in twenty-six weeks. Her basal metabolic rate before treatment was plus 6 per cent and reached a maximum of plus 88 per cent after the administration of 500 mg. of sodium dinitrophenol a day regularly for five weeks, the test being made one and one-half hours after the morning dose of 200 mg.

On June 12, 1934, the patient reported that for the previous two weeks she had noted a severe burning sensation in the balls of both feet and in all of the toes. Sharp, shooting pains were also experienced occasionally in these regions. Examination in the Neurological Clinic on July 3, 1934, by Dr. W. F. Beerman showed "a low stocking analgesia and anesthesia of the left foot extending from the external condyle downward including the whole of the foot. The toes were quite red. There was also hypalgesia to pin-prick on all of the toes and on the entire plantar surface of the right foot."

On July 7, 1934, she stated that her menstrual period was two weeks overdue. Her periods had previously been regular. A pelvic examination revealed that the uterus had the size, shape, and consistency of a six-week gestation. An Aschheim-Zondek Test on the urine on Aug. 7, 1934, was reported as being positive.

The dinitrophenol was discontinued on July 24, 1934, but was started again on Aug. 7, 1934. During the period in which she was not taking the drug, there was no marked improvement in the condition of her feet.

The patient entered Mount Zion Hospital on Aug. 22, 1934, having again been on dinitrophenol therapy for two weeks. At this time she said that she had been perfectly well, except for the burning sensations and pains in her feet, until the day before entry. There

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had been no symptoms of pregnancy other than the cessation of menses. At 11:40 A.M. on Aug. 21, 1934, she had helped lift a very heavy woman. At about 6:00 P.M. of the same day she noted a slight amount of vaginal bleeding and this was somewhat increased the next morning. However, she continued to take the regular doses of dinitrophenol during this time. At about noon she began to bleed profusely from the vagina and was still bleeding when she was brought into the hospital. Her underclothes were found to be soaked with blood. Examination showed her skin to be pale, cold, and clammy. The oral temperature was 36.4° C. (97.5° F.). The heart sounds were faint and the pulse was rapid and almost imperceptible. She was very short of breath and fainted several times before treatment could be instituted. The patient also complained of abdominal and precordial pains. Treatment was given for shock and hemorrhage and one hour after entry the uterus was completely emptied by means of a dilatation and curettage. No fetus was found. After the shock and hemorrhage had been controlled, the patient complained of feeling "hot." Dinitrophenol therapy was discontinued as soon as the patient entered the hospital and has not been started again.

The patient admitted that she did not wish to have this baby but on numerous occasions stated consistently that neither she nor anyone else had done anything to terminate the pregnancy.

Physical examination on Aug. 23, 1934, revealed an obese female of about thirty-five years of age. The temperature was 37.8° C. (100° F.), pulse 106 per minute, and respirations 24 per minute. The skin was warm and moist and both the skin and mucous membranes were pale. No palpable lymph nodes were found. Fairly marked hypertrichosis of the chin was present. The skull, eyes, ears, nose, mouth, and neck were not remarkable. The tonsils were present and slightly enlarged. The breasts were pendulous, the nipples were inverted and there was no increase in the pigmentation of the areolae. The lungs were clear and the heart was normal. The blood pressure was 100 systolic and 60 diastolic. The peripheral arteries were soft. The radial pulses were moderately weak, but were otherwise not remarkable. The abdomen was negative except that it was flabby and exhibited numerous striae. The abdominal reflexes were not obtained, probably because of the obese and atonic abdominal wall. Inspection of the external genitalia showed no evidence of continued hemorrhage.

Examination of the extremities revealed no abnormalities in the arms. The biceps and triceps reflexes were active and equal. Examination of the lower extremities showed analgesia of the soles of the feet and of the distal phalanges of all the toes. There was hypesthesia over the remainder of the dorsal aspect of the feet extending up along the inner side of both calves. The vibratory sense was absent at the ankles and knees but normal over all bony prominences above the pelvis. The deep muscle sense was entirely lost in the toes. The muscle power and coordination were normal. The patellar and achilles reflexes were very difficult to obtain but were present and equal. The plantar reflexes were normal and there was no clonus present.

Laboratory Findings.—The blood count on entry showed hemoglobin 79 per cent (Sahli), or 12.3 gm. per 100 c.c. of blood; red blood cells 4,320,000 per c.mm.; white blood cells 17,100 per c.mm. The differential count showed polymorphonuclears 74 per cent, eosinophiles 4 per cent, monocytes 5 per cent, and lymphocytes 17 per cent. Eighty-nine per cent of the polymorphonuclear leucocytes were filamented. The Wassermann and Kahn reactions on the blood serum were negative. On entry, the blood gave a positive test for alpha-dinitrophenol according to the method of Bolliger.[2]

The urine was negative except for a one-plus albumin reaction.

The spinal fluid on Aug. 24, 1934, showed no cells, a negative globulin test and a colloidal gold reaction of 0000000000. A test for alpha-dinitrophenol was negative.

Microscopic examination of the curettings from the uterus showed decidual reaction of the endometrium with formation of chorionic villi indicating pregnancy.

The basal metabolic rate on Aug. 26, 1934, was plus 1 per cent.

Subsequent History.—The patient was next seen in the Out-Patient Department on Sept. 6, 1934. At this time, although she still complained of some numbness in her feet, neurologic examination indicated a marked improvement as evidenced by the disappearance of most of the abnormal findings noted on Aug. 23, 1934.

Slight diminution to tactile and painful sensations over the soles of the feet were the only residual abnormal findings. This was two weeks after dinitrophenol had been discontinued. One week later she was normal both subjectively and objectively. There has been no return of symptoms to date.

TOXICITY OF ALPHA-DINITROPHENOL

Dinitrophenol may produce toxic effects as a result of overdosage or by doses within the normal therapeutic range in hypersensitive patients. Mayer (quoted by Perkins[3]) cites the fact that in munition factories, toxic manifestations develop in only a small number of workers despite the fact that others are working under identical conditions. He also states that chronic rheumatism, chronic alcoholism, tuberculosis, renal disorders, and hepatic disease decrease the tolerance of the patient to dinitrophenol.

Matzger[4] reported 157 patients on whom he performed skin tests with dinitrophenol. All gave negative reactions, but three later developed skin manifestations while under dinitrophenol therapy. This suggests that these tests will not be useful in detecting patients who are likely to develop a hypersensitivity to this drug. Frumess[5] reported an instance of a patient with a skin eruption due to dinitrophenol who gave positive scratch and passive transfer tests with dinitrophenol.

A review of the available literature failed to reveal any reports of patients suffering from fully developed peripheral neuritis or abortion due to dinitrophenol. The toxic effects vary greatly in each patient. Among those noted are asthenia, loss of weight, backache, pain in the chest, hyperpyrexia, feeling of warmth, excessive perspiration, night sweats, acidosis, coma, and even death.* It may also cause euphoria, mental confusion, headache, vertigo, and pains and paresthesias in the extremities. Its toxicity for the cardiovascular system may be manifested by tachycardia and fall in blood pressure. Dinitrophenol may cause pharyngitis and otitis media. The French have noted a type of shortness of breath which they termed "thermic dyspnea" as a complication of dinitrophenol administration. Vomiting also may be encountered. The effect on the liver may take the form of a toxic hepatitis with jaundice. Among the numerous possible skin complications may be mentioned urticaria, pruritic maculopapular erythema, hemorrhagic bullae, exfoliative dermatitis and jaundice. Dinitrophenol may also lead to agranulocytic angina. Hyperglycemia may also occur. Albuminuria has also been noted in patients taking dinitrophenol.

Several autopsies have been reported but there has been no uniformity of findings in these few cases.

DISCUSSION

The evidence presented strongly suggests that the peripheral neuritis in this patient was due to dinitrophenol. Numerous instances of patients suffering from pains and paresthesias in the extremities while on dinitrophenol medication have been reported.[12, 13] The neuritis developed in this patient while the drug was being taken and disappeared within three weeks after its administration was discontinued. Trinitrotoluene,[15] benzol[16] and other compounds chemically closely related to dinitrophenol have caused true peripheral neuritis. From this evi-

*For references to toxicity see [1,2,5 to 14].

dence it would seem justifiable to conclude, at least tentatively, that the neuritis in this patient was due to the dinitrophenol.

Deacon[17] in 1918 stated that it is not particularly dangerous for pregnant women to work in munition factories. However, in her series of 101 women who became pregnant while working in a munition factory, thirteen had early miscarriages. She attempts to explain only four of these. This is a high percentage and suggests the possibility of the existence of an unrecognized toxic agent as a causative factor in these miscarriages. Unfortunately no statement is made as to whether or not dinitrophenol was used in this munition factory.

A miscarriage could be due to the drug passing through the placenta and causing the death of the fetus, or the mechanism might be analogous to the early premature separation of the placenta sometimes seen in hyperthyroidism.[18] The absence of any discoverable etiologic factor except dinitrophenol in our patient makes it probable that the drug was responsible for the abortion. The importance of these two complications makes it advisable that the possibility of dinitrophenol causing peripheral neuritis and miscarriage be brought before the medical profession.

SUMMARY

1. A case is reported in which peripheral neuritis and abortion occurred in a patient while under dinitrophenol therapy.

2. A brief review of the problem of dinitrophenol toxicity is presented. This includes evidence which indicates the probable relationship between the administration of dinitrophenol and the occurrence of peripheral neuritis and miscarriage in the case presented.

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THE RESISTANCE OF RED BLOOD CELLS TO HEMOLYSIS IN HYPOTONIC SOLUTIONS OF SODIUM CHLORIDE*

Observations in Blood Disorders

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INTRODUCTION

THE osmotic resistance of the red blood cells has been of interest since 1883, when Hamburger[1] first developed a method of determining the susceptibility to hemolysis of the red blood cells in hypotonic concentrations of salt solution. Partly because there have been many modifications of technic employed by different investigators and partly because the observations have not always been made upon comparable stages of the same disease, inconstant results of the test in different disorders, particularly in anemia, have caused its value to be debated. The purpose of this investigation was, therefore, to study by means of a refined technic the resistance of the red blood cells to hypotonic salt solution in different disorders, especially in the same individual over a prolonged period of time. Furthermore, it was desired to discover if any relationship existed between resistance and the physical and chemical constitution of the red cells.

METHODS

Determination of the Resistance of Red Blood Cells.—One hundred cubic centimeters of stock salt solution were made from chemically pure sodium chloride for each 0.02 per cent concentration between 0.80 and 0.04 per cent inclusive, and kept in tightly stoppered bottles. One cubic centimeter of each concentration of sodium chloride was put into each of a series of appropriately labeled test tubes (10 mm. by 75 mm.). The tubes had previously been thoroughly cleaned without the use of acid.

Five cubic centimeters of venous blood were rendered incoagulable for the blood counts and resistance studies, using 0.05 c.c. of a 20 per cent solution of potassium oxalate. The cells of 2 c.c. of this sample were washed twice by adding 5 c.c. of 0.85 per cent sodium chloride to the whole blood, centrifuging twice at 1,500 revolutions per minute for fifteen minutes, and removing the supernatant fluid. The total volume was made up with 0.85 per cent sodium chloride to five times the volume of packed cells. One-tenth of a cubic centimeter of this 20 per cent cell suspension was added to each tube of salt solution and thoroughly mixed. The tubes were then allowed to stand from one to three hours in the refrigerator. Then all the tubes were packed into carriers and centrifuged for fifteen minutes at 1,500 revolutions per minute. The tubes were then arranged in order in a rack, and the points of hemolysis observed. Thus

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the technical variations were minimized by employing uniformly measured volumes of cells and sodium chloride solution, and by observing the end-points with particular care as described below.

Minimum Resistance.—Trace of hemolysis was determined by comparing the supernatant fluid in each tube in question with a control tube against a source of light with a blue daylight glass. The slightest color in the supernatant fluid was considered to indicate a *trace* of hemolysis. *Definite* hemolysis was determined by noting the highest concentration of sodium chloride in which an obvious reddish color appeared in the supernatant fluid.

Maximum Resistance.—Partial hemolysis was characterized by the persistence of a definite globule of cells or "button" in the bottom of the tube. The point of *complete* hemolysis was determined microscopically by observing the sediment in each tube below the "button." Hemolysis was considered complete when less than forty unhemolyzed red cells were present per field using a 40 mm. objective. The microscopic method of determining the end-point is of special value when the white blood count is high because leucocytes are much more resistant to hypotonic salt solution than are erythrocytes and, therefore, confuse the macroscopic observation of the end-point.

DETERMINATION OF HEMATOLOGIC DATA

Blood counts were done on the venous blood, using U. S. Bureau of Standards pipettes and counting chamber. The Sahli hemometer was standardized so that 100 per cent was equivalent to 21 volumes per cent of oxygen, assumed to be the capacity of 15.6 gm. of hemoglobin per 100 c.c. of blood. The Wintrobe hematocrit was used and indices determined.[2] The percentage of reticulocytes was determined by counting 1,000 red blood cells in smears of blood which had been brought in contact with brilliant cresyl blue in the moist state, fixed and stained with Wright's stain. The cell diameter measurements were made on fixed smears, using a projection apparatus according to the method of Price-Jones,[3] and the mean diameter and standard deviation calculated on 250 cells for each blood.

RESULTS

Resistance to Hemolysis in Hypotonic Solutions of Sodium Chloride of the Red Blood Cells in Blood Disorders.—More than 1,000 observations of red cell resistance have been made on some 200 individuals. A summary of some of these observations is presented in Table I. An average of the results for each group does not give an entirely correct picture because of the wide range of variation which has been observed in the same type of disease; therefore, the highest and lowest values (extremes) for each group, as well as the average, are given. In the cases of anemia the observations included in Table I were all made before any potent therapy was given.

Acute Blood Loss.—In the cases of acute blood loss, although the anemia was severe and some reticulocytes were present, the remaining red blood cells were practically normal and their resistance remained normal. The variation, as shown in Table I, was no greater than that in the normal individuals.

TABLE I

RESISTANCE TO HYPOTONIC SODIUM CHLORIDE SOLUTIONS OF THE RED BLOOD CELLS OF NORMAL INDIVIDUALS AND OF PATIENTS WITH VARIOUS BLOOD DISORDERS

DIAGNOSIS	NUMBER OF CASES	MEAN CORPUSCULAR VOLUME (CUBIC MICRA)		MEAN CORPUSCULAR HEMOGLOBIN CONCENTRATION (PER CENT)		PERCENTAGE CONCENTRATION OF SODIUM CHLORIDE IN WHICH HEMOLYSIS OCCURRED							
						MINIMUM RESISTANCE				MAXIMUM RESISTANCE			
						TRACE		DEFINITE		PARTIAL		COMPLETE	
		EXTREMES	AVERAGE	EXTREMES	AVERAGE	EXTREMES	AVERAGE	EXTREMES	AVERAGE	EXTREMES	AVERAGE	EXTREMES	AVERAGE
Normal	20	97-81	91.6	35-29	31.8	0.48-0.46	0.47	0.46-0.40	0.44	0.36-0.30	0.33	0.30-0.24	0.27
Acute blood loss	5	111-92	101.9	35-33	33.4	0.48-0.44	0.46	0.44-0.40	0.42	0.34-0.28	0.30	0.28-0.24	0.26
Hypochromic anemia													
Idiopathic hypochromic anemia with achlorhydria	17	76-51	65.4	29-20	25.4	0.60-0.48	0.52	0.52-0.42	0.50	0.32-0.18	0.28	0.26-0.10	0.19
Carcinoma of stomach with achlorhydria	2	80-57	68.5	28-24	26.2	0.54-0.48	0.51	0.52-0.46	0.49	0.24-0.18	0.21	0.20-0.16	0.18
Chronic blood loss	12	95-62	76.5	31-22	26.6	0.54-0.42	0.50	0.48-0.40	0.45	0.36-0.22	0.29	0.28-0.18	0.23
Poor diet and infection	3	83-73	78.5	27-25	26.1	0.54-0.48	0.50	0.50-0.44	0.47	0.30-0.24	0.26	0.22-0.16	0.18
Pregnancy	4	84-68	77.1	30-24	27.3	0.54-0.48	0.50	0.52-0.46	0.48	0.30-0.20	0.27	0.26-0.16	0.22
Thrombocytopenic purpura	3	94-73	83.6	34-24	29.2	0.52-0.46	0.48	0.50-0.40	0.44	0.34-0.28	0.31	0.26-0.18	0.23
Hemophilia	2		84.4		30.3		0.46		0.42		0.32		0.27
Erythroblastic anemia (Cooley's)	5	117-68	92.4	33-23	27.7	0.76-0.50	0.58	0.60-0.46	0.49	0.26-0.16	0.20	0.18-0.08	0.13
Sickle cell anemia	1		93.1		30.4		0.46		0.40		0.22		0.10
Pernicious anemia	20	153-91	118.5	34-25	30.8	0.60-0.42	0.49	0.50-0.38	0.44	0.36-0.26	0.32	0.30-0.24	0.27
Macrocytic anemia of pregnancy	4	107-88	101.7	34-22	27.4	0.54-0.48	0.51	0.50-0.44	0.47	0.38-0.36	0.37	0.34-0.26	0.29
Osteosclerotic anemia	1		101.1		29.4		0.52		0.48		0.32		0.28
Aplastic anemia	2	126-91	108.6	34-27	31.0	0.48-0.46	0.47	0.44-0.42	0.42	0.36-0.34	0.35		0.30
Polycythemia rubra vera	5	115-59	88.8	38-32	32.6	0.60-0.46	0.51	0.50-0.42	0.46	0.36-0.28	0.32	0.30-0.20	0.26
Chronic hemolytic jaundice													
Before splenectomy	13	104-70	88.2	38-30	34.5	0.85-0.50	0.76	0.80-0.48	0.62	0.44-0.24	0.38	0.42-0.18	0.28
After splenectomy	3	96-81	86.8		35.5	0.70-0.64	0.66	0.62-0.60	0.60		0.36		0.29
Chronic lymphatic leucemia	3	107-89	96.0	32-30	31.2	0.52-0.48	0.50	0.50-0.46	0.48	0.36-0.34	0.35		0.30
Chronic myelogenous leucemia	7	103-77	92.0	34-27	30.6	0.80-0.50	0.58	0.60-0.46	0.53	0.36-0.24	0.33	0.32-0.22	0.28

Hypochromic Anemia.—The blood picture of hypochromic anemia is characterized by small cells poorly supplied with hemoglobin, showing considerable variation in size and shape, whether associated with bad diet, gastric anacidity, blood loss or pregnancy. An increased span of resistance, especially with increased maximum resistance, has been observed by Minot,[4] Waugh,[5] and Scotti-Douglas and Dondi.[6] The association of increased resistance with microcytosis has been demonstrated by Scotti-Douglas and Dondi. Graham[7] emphasized the fact that the diminished minimum resistance in these cases is similar to that found in hemolytic jaundice and, on that basis, suggested that there is a relationship between the two diseases. He did not observe an increased maximum resistance.

The most characteristic alteration of red cell resistance in the group of thirty-eight patients with hypochromic anemia (recorded in Table I) was the increased maximum resistance of the cells. A decreased minimum resistance also occurred in some cases. The first group included seventeen women with idiopathic hypochromic anemia with achlorhydria. In ten of these complete hemolysis occurred in 0.20 per cent sodium chloride solution or below. Five of the patients had the syndrome of hypochromic anemia, dysphagia, and glossitis. In contrast to the report of Graham[7] the maximum resistance was increased in nearly all, which is contrary to the usual finding in chronic hemolytic jaundice.

In chronic blood loss, whether due to menorrhagia, hemorrhoids, carcinoma, thrombocytopenic purpura or hemophilia, the characteristic increased maximum resistance was found when the blood values were such as obtain in other types of hypochromic anemia. In four cases of hypochromic anemia associated with pregnancy and three cases with poor diet and infection, the resistance of the red cells was similar to that of other types of hypochromic anemia and apparently varied only with the severity of the anemia. On the whole, the span of resistance was not so great in these groups of cases as in those which had achlorhydria.

Erythroblastic Anemia (Cooley's).—In erythroblastic anemia which has been described recently by Cooley and Lee[8] and by Baty, Blackfan, and Diamond[9] there is an increased maximum resistance of the red blood cells, but there is a difference of opinion in regard to alterations in the minimum resistance. Observations of the resistance of the red blood cells in five patients (Table I) showed that the span of resistance was much greater in all than in normal individuals. The minimum resistance was decreased in three cases but the most striking change occurred in the maximum resistance. Complete hemolysis occurred in 0.14, 0.18, 0.10, 0.16, and 0.08 per cent sodium chloride solution, respectively. The increased maximum resistance thus resembles that found in severe hypochromic anemia. Observations on one case of sickle-cell anemia showed a similar increased maximum resistance.

In erythroblastic anemia, a type of hypochromic anemia probably not related to iron deficiency, the red cells show marked achromia with extreme variation in size and shape. A special feature of this type of blood is the presence of poikilocytes. The mean diameter of the cells was normal, below or above normal, but the deviation of the diameters from the mean and the coefficient of variation were high (standard deviation = 1.20 and 1.19, and coefficient of variation

= 17.10 per cent and 18.42 per cent). The marked increase in maximum resistance occurred in the same bloods which showed a great many small cells and poikilocytes which remained unhemolyzed in the dilute solutions.

Pernicious Anemia.—Although the resistance of the red blood cells in pernicious anemia in relapse varies much more than in normal individuals, the average values for maximum and minimum resistance have been reported[10, 11, 12] as very close to that of normal individuals. In twenty cases studied before treatment the abnormal feature was found in the minimum resistance. The trace of hemolysis either did not appear at all until there was color enough to be seen at a glance, and thus coincide with the point for definite hemolysis, or there was slight coloring of the supernatant fluid in several of the tubes above the point of definite hemolysis. This trace of hemolysis, due no doubt to the destruction of a few red cells, has often been overlooked or disregarded. The test is not of diagnostic value for pernicious anemia, because of the normal span of resistance in many cases and the wide range of variation. The resistance of the red cells of four patients with the pernicious (macrocytic) anemia of pregnancy gave similar results to those found in Addisonian pernicious anemia.

Osteosclerotic Anemia.—Occasional observations were made on a patient with osteosclerotic anemia recently reported by Chapman.[13] The patient had between 1 and 2 million red blood cells per cubic millimeter for more than a year. The resistance of the red cells was studied about two months after each of several transfusions. The minimum resistance was decreased throughout. The mean corpuscular volume was slightly above normal most of the time, while the mean corpuscular hemoglobin concentration was below normal. There were 5 to 10 per cent of reticulocytes present throughout this period.

Aplastic Anemia.—Increased minimum and a normal or decreased maximum resistance have been recorded as a feature of advanced aplastic anemia.[14, 15, 16] The consistent change has been stated to be a decreased maximum resistance and increased minimum resistance, producing a narrow span of resistance. Observations in the late stages of the disease of two patients showed a slightly decreased span of resistance.

Polycythemia Rubra Vera.—The resistance of the red cells in polycythemia rubra vera has been discussed by Minot and Buckman.[17] The variability reported by them was confirmed by the present study of five patients. In two patients the minimum resistance was 0.56 and 0.60 per cent sodium chloride, respectively, while the maximum resistance was practically normal. In other patients the minimum resistance was normal and the maximum resistance was increased, or a normal resistance was present. These differences may be manifestations of the varied red cell values which obtained in these patients.

Chronic Hemolytic Jaundice.—A study of thirteen cases of chronic hemolytic jaundice confirmed the observations of previous authors.[18, 19, 20, 21] A decrease in the minimum resistance was found in all these cases. The maximum resistance was decreased in eleven of the thirteen cases. The abnormal resistance of the red blood cells to hypotonic sodium chloride solution was not found to be an index of the severity of the anemia or of the clinical condition of the patient.

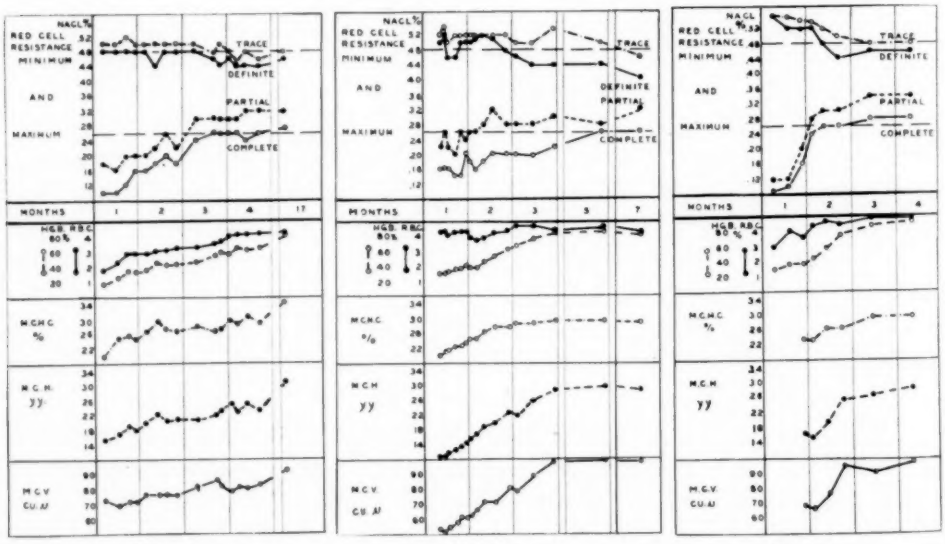
Observations on three patients with hemolytic jaundice were made one, two, and five years, respectively, after splenectomy. These patients were symptomatically well and the blood counts were normal, but the resistance, both minimum and maximum, was still decreased. This agrees with the work of other investigators that the resistance to hypotonic salt solution remains abnormal after splenectomy, although the number of reticulocytes becomes normal, icterus disappears and the clinical condition of the patient becomes greatly improved.

Leucemia.—Three cases of *chronic lymphatic leucemia* showed practically a normal span of resistance. At times during eight months of observation in one case the minimum resistance was decreased to 0.52 and 0.50 and the maximum resistance varied from 0.26 to 0.34 per cent of sodium chloride at different times. Complete hemolysis was very abrupt, as confirmed by direct microscopic examinations of the sediment. The resistance of the red cells in *chronic myelogenous leucemia* varied within wide limits. In the seven patients studied the minimum resistance was decreased. There seemed to be no definite correlation in these cases between the resistance span and the number of the red cells, or the number and age of the white cells.

Progressive Changes in the Resistance of the Red Blood Cells of Patients During Treatment.—Progressive changes in the resistance to hemolysis of the red cells were observed in certain types of anemia during a period of weeks or months while treatment was being given. As the degree of anemia changed there were changes in many of the corpuscular values; for example, in the mean corpuscular volume and hemoglobin content, and in the resistance to hemolysis. The consideration of the stage of the disease, therefore, is important in studying the resistance of the red blood cells.

Hypochromic Anemia.—In hypochromic anemia the abnormal resistance of the red cells approached normal values as the small cell volume and lowered hemoglobin concentration increased toward normal as the result of effective therapy. These characteristic changes are illustrated by observations upon three patients with hypochromic anemia made during the period of response to therapy with iron and are presented graphically in Fig. 1. The first case (Case 1) was a patient who had idiopathic hypochromic anemia with glossitis, dysphagia, and achlorhydria. The red blood cell count on admission was 1,900,000 per c.mm. and the hemoglobin was 19 per cent. The maximum resistance was increased, complete hemolysis occurring in as low a concentration as 0.10 per cent sodium chloride. The minimum resistance was only slightly diminished. The volume of the red blood cells was below normal (75 c. micra) and the mean corpuscular hemoglobin concentration was low (20.1 per cent). As the mean corpuscular volume and hemoglobin concentration rose in response to the daily administration of iron and ammonium citrate, the point of maximum resistance or complete hemolysis approached the normal value. The red cells also increased in thickness as the concentration of hemoglobin increased (Table II). When first observed, the percentage of poikilocytes in the blood smears of this patient was 14.6 per cent, but four months later, when the red cell count, hemoglobin value, and resistance were nearer normal, this percentage was 6.6. The size of the

cells in a given sample also varied markedly. The standard deviation of the diameters of the cells and the coefficient of variation have been determined to show the heterogeneity of the cell population (Table II). This standard deviation is a measure of the dispersion of the diameters, their range in size, and the way in which the numerical frequencies of the diameters are arranged.[3] These values are very much increased in these severe hypochromic anemias, but return toward normal, showing greater uniformity of the cells coincident with the return of the resistance values to normal. Direct observations of the nonhemolyzed erythrocytes in the tubes of salt solution near the point of complete hemolysis indicated that the change from partial hemolysis (the tube in which the "button" of cells appears) to complete hemolysis was more gradual than that which occurred in the blood of normal individuals. When the sediments in the tubes



Case 1. Case 2. Case 3.

Fig. 1*.—Resistance to hemolysis in hypotonic solutions of sodium chloride of the red blood cells in hypochromic anemia.

*In Figures 1 to 4 inclusive the following abbreviations are used:
R.B.C., red blood cells in millions per c. mm.
Hgb., hemoglobin expressed in per cent; 100 per cent=15.6 gm.
M.C.H.C., mean corpuscular hemoglobin concentration in per cent.
M.C.H., mean corpuscular hemoglobin in micromicrograms (γγ)
M.C.V., mean corpuscular volume in cubic micra (cu. μ)
M.C.D., mean corpuscular diameter in micra (μ)
M.C.T., mean corpuscular thickness in micra (μ)
Red Cell.
Resistance, The average normal value for minimum resistance (trace of hemolysis) and maximum resistance (complete hemolysis) is shown by dash lines on each chart.

of salt solution of low concentration were observed under the microscope, polkilocytes and small cells represented a large percentage of the nonhemolyzed cells.

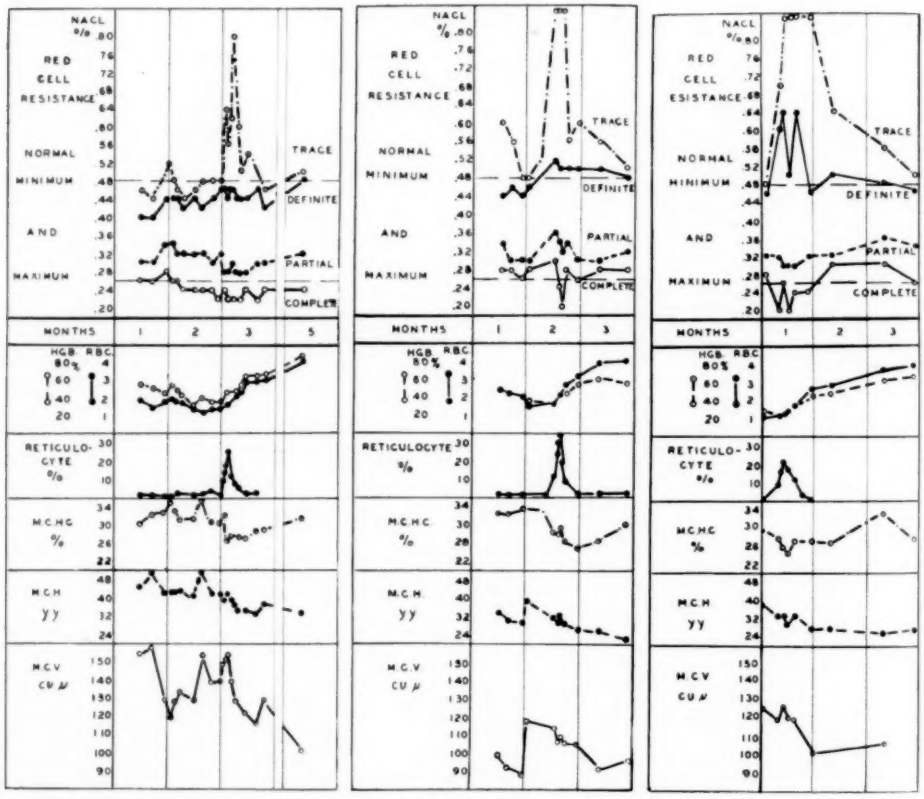
In the second case of idiopathic hypochromic anemia the initial red count was 4,350,000 per c.mm. and the hemoglobin, 31 per cent, which represents a very low mean corpuscular hemoglobin. The maximum resistance was in-

TABLE II

CHANGES DURING TREATMENT IN THE SIZE OF THE CELLS AND RESISTANCE TO HYPOTONIC SODIUM CHLORIDE SOLUTIONS OF THE RED BLOOD CELLS IN TWO PATIENTS WITH HYPOCHROMIC ANEMIA

	R. B. C. (MILLIONS)	HEMOGLOBLIN (PER CENT)	RETICULOCYTES (PER CENT)	M. C. V. (C. MICRA)	M. C. D. (MICRA)	STANDARD DEVIATION OF DIAMETER	COEFFICIENT OF VARIATION (PER CENT)	M. C. T. (MICRA)	PERCENTAGE CONCENTRATION OF SODIUM CHLORIDE IN WHICH HEMOLYSIS OCCURRED			
									MINIMUM RESISTANCE		MAXIMUM RESISTANCE	
									TRACE	DEFINITE	PARTIAL	COMPLETE
Case 1												
1/29/32	2.9	35	0.9	73.2	7.44	1.08	14.5	1.67	0.50	0.48	0.20	0.12
2/12/32	3.2	46	0.5	76.3	7.31	1.06	14.5	1.81	0.50	0.44	0.22	0.18
3/21/32	3.7	55	1.0	86.0	7.42	0.86	11.59	1.99	0.48	0.46	0.30	0.26
4/ 6/32	4.1	66	1.2	82.1	7.52	0.75	9.96	1.84	0.44	0.44	0.30	0.26
4/22/32	4.1	65		83.0	7.24	0.64	8.83	2.02	0.46	0.44	0.32	0.26
Case 2												
7/13/32	4.3	31	1.6	51.7	6.33	0.83	13.1	1.64	0.54	0.52	0.26	0.16
7/21/32	4.4	36	1.2	57.1	6.68	0.89	13.32	1.63	0.52	0.46	0.20	0.14
8/10/32	3.8	46	12.2	70.6	7.65	0.97	12.67	1.54	0.52	0.52	0.28	0.18
8/17/32	4.2	53		70.5	7.67	1.12	14.6	1.53	0.52	0.52	0.32	0.20
10/23/34	4.5	91		93.8	7.60	0.65	8.55	2.06	0.48	0.44	0.34	0.30

creased to 0.16 per cent sodium chloride, returning to normal with the return of the red cell and hemoglobin values to normal. The minimum resistance was slightly decreased during the first three months of therapy and then returned to normal. During this period the mean corpuscular volume, which was unusually low (53 c. micra), doubled and the mean corpuscular hemoglobin returned to normal. The standard deviation of the red cell diameters from the mean and the coefficient of variation on this patient are also shown in Table II. These values showed some variation during the reticulocyte response but became lower, showing a decrease in the heterogeneity of the cells, as the span of resistance and other red cell values returned to normal.



Case 4. Case 5. Case 6.

Fig. 2.—Resistance to hemolysis in hypotonic solutions of sodium chloride of the red blood cells in pernicious anemia.

The third patient presented in Fig. 1 had the microcytic hypochromic anemia of pregnancy and was first observed following delivery. Here the return of the resistance span to normal values was much more abrupt than in the other cases because of the rapid rise of blood values due to intensive iron therapy. During the first month following delivery the maximum resistance was increased markedly, as in the other cases. The minimum resistance was decreased during the first two months and returned to normal when the hemoglobin concentration had reached 30 per cent. The mean corpuscular volume

rose from 65 cubic micra to over 90 cubic micra coincident with the return of the maximum resistance to normal.

Pernicious Anemia.—Observations made upon three patients with pernicious anemia are presented in graphic form in Fig. 2 and serve to illustrate the typical phenomena in relation to changes in resistance occurring in liver-induced remissions in this disease. Since the original abnormality of the resistance in this condition is not so striking as in hypochromic anemia, the progressive changes occurring with improvement are not so striking as are those associated with the reticulocyte responses. Both minimum and maximum resistance may be altered at the time of increased production of young red cells. Thus, in each of these patients the reticulocyte response to effective liver therapy was accompanied by a decreased minimum resistance. Not only was the point of trace of hemolysis altered at the time of the reticulocyte response, but also the point of definite hemolysis. The occurrence of definite hemolysis in such high concentrations means that a great many of the cells became less resistant. Co-

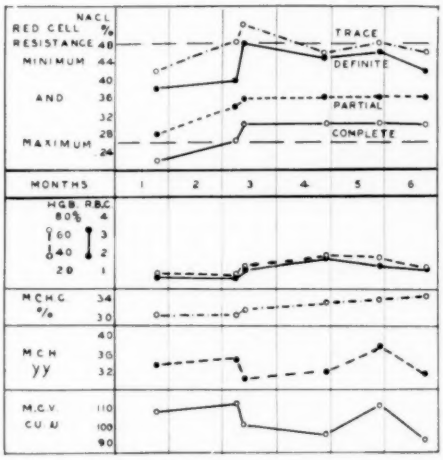


Fig. 3.—Case 7. Resistance to hemolysis in hypotonic solutions of sodium chloride of the red blood cells in aplastic anemia.

incident with the reticulocyte response and the decrease in the minimum resistance there was a sudden decrease in mean corpuscular hemoglobin and hemoglobin concentration. Some increase in the mean corpuscular volume also occurred. Since the new young cells (reticulocytes) are usually larger than those normally present in the blood stream[23, 24, 25] but relatively poor in hemoglobin, it is suggested that such cells may be responsible for the changes observed during the response to liver extract therapy. The minimum resistance was, however, not always decreased at the time of the reticulocyte response. Under these circumstances it was found that the mean concentration of hemoglobin was normal. After the red cells had reached at least a level of 4,000,000 per c.mm., the maximum and minimum resistance were normal.

The maximum resistance was practically normal in all three patients before treatment. There were marked increases in the maximum resistance at the time of the reticulocyte responses. A slight increase in the maximum resistance

developed in Case 4 during and after treatment. Coincident with this increased maximum resistance there was a decreased concentration of hemoglobin in the cells. Thus these cells became low in hemoglobin like the cells in the less severe cases of hypochromic anemia in which comparable changes in the maximum resistance occurred.

Aplastic Anemia.—The observations on two patients with aplastic anemia seem to show correlations between the normal or decreased span of resistance and the relative uniformity of the red cells. The red blood cells of both patients remained below 2,500,000 per c.mm. throughout the period of observation. The data from observations upon one of these cases are shown in Fig. 3. The span of resistance was decreased in four out of six observations. It may be seen

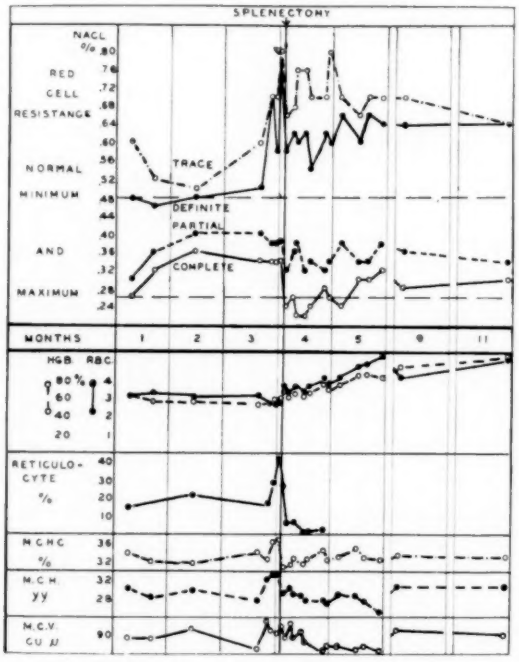


Fig. 4.—Case 8. Resistance to hemolysis in hypotonic solutions of sodium chloride of the red blood cells in chronic hemolytic jaundice.

that the difference between definite and partial hemolysis is very much less than normal in this case. The mean corpuscular hemoglobin concentration in this case was about normal, while the mean corpuscular volume and the mean corpuscular hemoglobin were greater than normal. The second case was similar in that the span of resistance was decreased in nine out of fourteen observations.

Chronic Hemolytic Jaundice.—Certain changes in resistance in hemolytic jaundice were observed in following the course of the individual cases. In Fig. 4 are presented data from a patient with congenital hemolytic jaundice who was studied for more than a year, during which time splenectomy was performed. Before splenectomy the minimum resistance varied from 0.50 to 0.80 per cent sodium chloride, and the maximum resistance, from 0.26 to 0.36 per

cent sodium chloride. Thus the span of resistance was less than normal in some observations. The hemoglobin content and volume of the red blood cells varied comparatively little during these three months until the last week before splenectomy. At this time a hemolytic crisis occurred and the reticulocytes increased from about 20 to 43 per cent, and the minimum resistance decreased to 0.80 per cent sodium chloride. The reticulocytes returned to normal almost immediately after splenectomy but the minimum resistance remained abnormal—a fact which has been observed by other investigators.[19, 20, 21] The markedly decreased minimum resistance fluctuated between 0.80 and 0.66 per cent sodium chloride during the first month after splenectomy, and at the end of seven months was still decreased to as much as 0.64 per cent sodium chloride. The maximum resistance is said to be increased after splenectomy, but, in this case, it remained increased only while the hemoglobin concentration was below normal. Following splenectomy there was a sudden relative decrease in the mean corpuscular hemoglobin concentration and gradual decrease in the mean corpuscular volume accompanied by an abnormally increased maximum resistance which persisted for nearly a month. Here, perhaps, is a resemblance to the conditions found in hypochromic anemia.

DISCUSSION

The greatest difficulty in determining from these observations what characteristics of the erythrocytes produce a given change in resistance is that the cell population is extremely heterogeneous in most pathologic conditions. To compare the end-points of a series of observations upon resistance with the mean values for cell volume and hemoglobin concentration is obviously not satisfactory. The breadth of the span of resistance was suggested by Chauffard[18] to have a relationship to the range of variation in the cell population. In order to demonstrate this variation a study of the standard deviation of the diameter in different types of cases has been made. It is not intended to imply that variation in diameter is the factor directly responsible for variation in resistance. Such a measurement is merely a practicable means of expressing heterogeneity of the cell population. In respect to resistance there may be some factor of heterogeneity of cell population less easy to measure. The standard deviation is a measure of the dispersion of the diameters, their range in size, and the way in which the numerical frequencies of the diameters are arranged. These figures have been determined for about thirty cases, including normal individuals, patients with pernicious anemia, hypochromic anemia, Cooley's anemia, hemolytic jaundice, acute blood loss, and aplastic anemia. In general, the greater the dispersion of the cell diameters, the greater is the span of resistance. The results on cases of hemolytic jaundice and pernicious anemia at the reticulocyte response seem to be exceptions. The progressive narrowing of the span of resistance in hypochromic anemia during treatment follows the same law. Examination of the blood smears of the cases which had a wide span of resistance also showed considerable variation in the amount of hemoglobin in different cells. The fact that a widened span of resistance in hypochromic anemia was often associated with increased maximum resistance and a more gradual appearance of the end-point is probably also an expression of greater heterogeneity. On

the other hand, in aplastic anemia the narrowed span of resistance may well be associated with the greater conformity to a given cell type, as was shown by the standard deviation of the diameters.

In 1922, Gänsslen[25] stated his belief that the cells in chronic hemolytic jaundice are more susceptible to hemolysis because they are more spherical than normal erythrocytes. According to Haden[26] this principle applies to other pathologic conditions in man, especially hypochromic anemia. In the blood of hypochromic anemia the presence of numerous poikilocytes (nonspherical cells) in the sediments in the lower concentrations of salt beyond the point of "button" is in accord with this principle. It is probable that the low hemoglobin concentration is an additional factor working for increased maximum resistance, since if the cells are not well filled with hemoglobin, the thickness of the cells might conceivably be decreased.

Although a lowered mean corpuscular volume and mean corpuscular hemoglobin concentration are also correlated with an increased maximum resistance in hypochromic anemia, Cooley's anemia, and polycythemia, and although the normal maximum resistance in pernicious anemia and aplastic anemia is associated with more normal values for mean corpuscular volume and hemoglobin concentration, it is probable that these correlations appear because of their influence on cell shape.

Reticulated, as well as nonreticulated, cells show physical changes which may be related to the changes in the resistance. By some investigators the increased maximum resistance is associated with increased reticulocytes, while by others the decreased minimum resistance is correlated with the increased reticulocytes. Simmel[27] gave a comprehensive review of the literature and concluded that difference in age of cells may cause this variation in resistance. Minot and Buckman[17] associated the increased span of resistance in erythremia with different ages of red cells in the blood. Either the minimum or the maximum resistance or both may be altered with the increase of reticulocytes, but there is no definite correlation between the actual number of reticulocytes in the blood and either the minimum or maximum resistance, if all types of cases are studied as one group. A study of the diameters of the cells in fixed preparations showed that the reticulocytes in different conditions or stages of the same conditions vary markedly in size from reticulated macrocytes to reticulated microcytes, and that the amount of hemoglobin in these cells also varied considerably. The best explanation that can be given at this time is that the sudden physical changes of the red cells in pernicious anemia at the time of the reticulocyte response, which are demonstrably associated with increase of the mean corpuscular volume or lowering of the mean corpuscular hemoglobin concentration, may change the resistance of the erythrocytes. These alterations in resistance are apparently not due to any quality inherent in the age of the red blood cells but to changes in their size, shape, hemoglobin concentration, or other factors.

SUMMARY

1. A method has been described for determining the resistance of the red blood cells to hypotonic salt solution. The amount of solution and the amount of

red cell suspension are kept uniform throughout. The careful reading of the end-points makes it possible to observe slight variations in the resistance of the red cells.

2. The usual observations of the maximum and minimum resistance in the various blood disorders may be summarized as follows:

Normal maximum resistance occurs in pernicious anemia and lymphatic leucemia.

Increased maximum resistance may be found in various sorts of hypochromic anemia, erythroblastic anemia (Cooley's), polycythemia rubra vera, and in myelogenous leucemia, depending upon the degree of anemia present. In idiopathic thrombocytopenic purpura and hemophilia the changes are apparently consistent with the degree of hypochromic anemia. After splenectomy in hemolytic jaundice and occasionally in pernicious anemia during treatment, a temporary hypochromic blood picture may be accompanied by increased maximum resistance.

Decreased maximum resistance is usually found in hemolytic jaundice and in advanced aplastic anemia.

Normal minimum resistance may be found in moderate degrees of hypochromic anemia, including that of purpura, hemophilia, Banti's disease, sometimes in pernicious anemia and in polycythemia vera. Rarely it is normal in hemolytic jaundice.

Increased minimum resistance occurs in pernicious anemia in severe relapse and in aplastic anemia.

Decreased minimum resistance is usually found in hemolytic jaundice and may be found in hypochromic anemia, erythroblastic anemia, polycythemia vera, and myelogenous leucemia.

3. In general, with the exception of hemolytic jaundice and pernicious anemia in remission, the standard deviation of the cell diameters increases as the span of resistance increases. This variation in cell diameter is somewhat representative of the heterogeneity of the cell population and may be better correlated with variations in resistance than mean values. In hypochromic anemia, as the standard deviation of cell diameters from the mean decreases as a result of therapy, there is a progressive decrease in the span of resistance.

4. The increased maximum resistance in hypochromic anemia, Cooley's anemia, and polycythemia rubra vera is associated with a lowered mean corpuscular volume and mean corpuscular hemoglobin concentration. The latter may be important in causing flattening of the erythrocytes observed by Haden[26] in this condition. According to the theory of Gänsslen[25] the numerous poikilocytes should be an additional factor making for increased resistance, since they present striking deviations from the spherical form associated with a decreased resistance in hemolytic jaundice.

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A CORRELATION OF ANATOMICAL FINDINGS WITH ABNORMALITIES OF THE Q_3-WAVE*

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CONSIDERABLE attention has been directed recently to disturbances of the initial part of the ventricular complex in the electrocardiogram, particularly a deep Q-wave in Lead III. Such a wave is a normal feature in cardiographs exhibiting right axis deviation. However, a great deal of discussion has been aroused as to its possible pathologic significance in cases with normal axis or left axis deviation. Parkinson and Bedford,[1] and Levine and Brown[2] reported the finding of a deep Q-wave in Lead III of the electrocardiogram in cases of coronary occlusion. Pardee[3] and Willius[4] found a deep Q-wave in Lead III in a variety of clinical conditions, notably anginal syndrome, arterial hypertension, arteriosclerosis with myocardial fibrosis, and pregnancy.

From their anatomical studies, and for theoretical reasons discussed in their paper, Fenichel and Kugell[5] concluded that injury to the smaller subdivisions of the conducting mechanism in the posterior part of the septum is probably responsible for the development of the characteristic Q-wave. Other authors,[6-8] on the other hand, believe that a deep Q-wave in Lead III can often be explained by a change in the axis of the heart. Wilson, et al.,[9] and later Barnes[10] have recently called attention to the frequency of a deep Q_3-wave in records showing inversion of T_3 in cases of infarction of the basal portion of the posterior wall of the left ventricle. Such records were designated Q_3T_3 types and were considered by Barnes to be more definitely correlated with basal ventricular infarction than T_3 changes alone. Since the posterior portion of the septum is frequently involved in cases of infarction of the left ventricle posteriorly, the question arises whether the deep Q_3 is correlated (1) solely with the septal pathology, as stated by Fenichel and Kugell or (2) with the ventricular wall lesion, which may or may not involve the septum posteriorly. Wilson, et al., and later Barnes further noted the presence of deep Q_1-waves in association with T_1 inversion (Q_1T_1 types) in cases of anterior wall infarction, also involving, as a rule, the anterior portion of the septum.

With the hope of throwing some light on this subject, an attempt is made in this report to correlate abnormalities of the Q-wave and inversion of the T-wave with pathologic findings in the heart, in a series of autopsies that have come under our observation during a period of about two years.

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Pardee's criteria for an abnormal Q-wave were as follows: An initial negative wave in Lead III of more than 25 per cent of the maximum deflection in any lead, immediately followed by a definite upright wave. In the present study, Pardee's criteria were followed except that those cases were also included which did not show a definite upward deflection immediately following the initial negative wave in Lead III (Fig. 2). Thus, cases with left axis deviation without an upward deflection at the beginning of the ventricular complex were considered abnormal by us. As has been pointed out by Shookhoff and Douglass, such an abnormal Q_3-wave does not correspond in time with Q_1 or Q_2 but is synchronous with parts of R_1 or R_2. This is to be expected since the wave in question is very frequently a Q-wave combined with the main

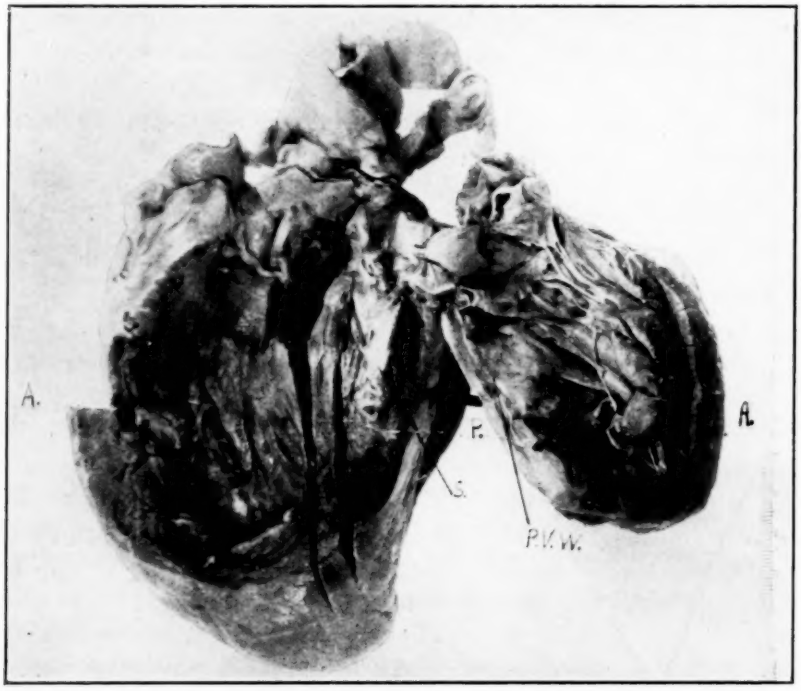

Fig. 1.—Heart (Case 1815) showing old infarctions of posterior ventricular and posterior septal walls. *A*, Anterior aspect. *P*, Posterior aspect. *P. V. W*, Posterior ventricular wall with area of marked thinning and scarring. *S*, Extension of scarred area to posterior portion of septum.

downward deflection. We hesitate to add a new term to the nomenclature, and, therefore, we have designated the combined deflection as a deep Q_3-wave or as an abnormal Q-wave.

Fenichel and Kugell described two cases with L.V.P. in which the initial upward deflection in Lead III was less than 5 per cent of the maximum total deflection. Both of these cases showed pathologic changes similar to those in which they found a deep Q-wave, and they called this particular type of excursion an indeterminate Q-wave. This type of wave was encountered once in our study.

Of thirty-two pathologic hearts with recent electrocardiograms available (taken within a month of death), we discarded five cases with bundle-branch

TABLE I

CASES WITHOUT OLD OR RECENT MYOCARDIAL INFARCTION

CASE	AUTOPSY NO.	PATHOLOGIC FINDINGS			ELECTROCARDIOGRAPHIC FINDINGS	
		CORONARY ARTERIES	VENTRICULAR WALLS	SEPTUM	INVERSION OF T-WAVE	ABNORMALITIES OF INITIAL COMPLEX
1	1598	Left coronary slightly sclerotic	Microscopic congestion and diffuse fibrosis	Microscopic congestion and fibrosis	None	None
2	1629	Arteritis both coronaries	Interstitial myocarditis	None	T_2	None
3	1650	Normal	Microscopic fibrosis at apex	None	None	None
4	1671	Both coronary arteries sclerosed but patent	Diffuse microscopic fibrosis (sclerosis of aorta)	Diffuse microscopic fibrosis	None	None
5	1679	Both extremely sclerotic lumen constricted	Diffuse myocarditis	Diffuse myocarditis	T_1	None
6	1692	Both sclerosed, patent	Intramuscular fibrosis (syphilis of aorta and aortic valve)	None	None	None
7	1700	Patent	Fibrinous pericarditis (terminal nephritis)	None	T_1 (T_2 isoelectric)	None
8	1738	Both thickened but patent	Hypertrophy. Hyaline changes	Hyaline changes	T_1	None
9	1609	Normal	Carcinomatosis of pericardium	None	T_1 (T_2 diphasic)	None
10	1600	Normal	Rheumatic pancarditis; diffuse myocarditis	None	T_1	None
11	1649	Normal	Pericarditis, endocarditis, diffuse interstitial myocarditis	None	T_2, T_3	None
12	1672	Normal	Rheumatic pancarditis	Endocardial roughening	T_1	None
13	1944	Normal	Chronic fibrous pericarditis	None	None	None
14	1691	Normal	Bacterial endocarditis. Diffuse suppurative myocarditis	Diffuse suppurative myocarditis	T_3	None

TABLE II

CASES OF OLD OR RECENT INFARCTION

CASE	AUTOPSY NO.	CORONARY ARTERY PATHOLOGY	PATHOLOGY OF VENTRICULAR WALLS	REGION OF VENTRICULAR WALLS INVOLVED	SEPTAL PATHOLOGY	ELECTROCARDIOGRAPHIC FINDINGS		
						INVERTED T-WAVE	ABNORMALITY OF INITIAL COMPLEX	AXIS
1	1660	Old thrombosis right coronary, left circumflex and left descending	Infarction, myomalacia and fibrosis anteriorly and posteriorly	Anterior and posterior	Infarction, myomalacia and fibrosis, anteriorly and posteriorly	None	Deep Q_3	L. A. D.
2	1792	Recent occlusion right coronary. Left anterior descending a fibrous cord. Left circumflex narrowed	Area of recent infarction posteriorly near base. Old infaret, fibrotic, anteriorly	Anterior and posterior	Necrosis and scarring posteriorly	None	Deep Q_3	L. A. D.
3	1815	Occlusion right coronary, left circumflex thrombosed; left descending occluded	Infarction, fibrotic, posteriorly near base. Suppurative myocarditis (traumatic rupture of heart)	Posterior	Infarction upper part posterior	T_3	Deep Q_3	L. A. D.
4	2057	Right circumflex occluded by organized and calcified thrombus	Myomalacia posteriorly, involving left ventricles and extending into right	Posterior	Myomalacia posteriorly	T_3	Deep Q_3	Normal axis
5	1633	Left descending thrombosed	Infarction anteriorly at apex	Anterior	Infarction of anterior third and of lower part of posterior septum	T_1, T_2	Deep Q_1	L. A. D.
6	992	Left descending occluded	Infarction anteriorly at apex	Anterior	Infarction lower half of septum anteriorly and posteriorly; adherent thrombus	T_1	Deep Q_3	Normal axis
7	970	Both coronaries occluded at ostia; free below	Infarction, fibrosis, necrosis anteriorly and posteriorly. Syphilitic aortitis	Anterior and posterior	Necrosis of entire septum	None*	Deep Q_3 (not quite 25%) Transient	Normal axis
8	1685	Left circumflex occluded	Infarction posterior wall of left ventricle	Posterior	Infarction upper part of posterior septum	None	Upward deflection, 3%	L. A. D.
9	1625	Occlusion descending branch of left coronary	Infarction of apex	Anterior	Infarction of anterior third of septum. Posterior septum free	T_1	None	L. A. D.

*Two later cardiographs showed inversion of T_1 and T_2 with no abnormal Q-wave.

block and four with right axis deviation. The twenty-three hearts which remained for study were divided into two groups, those without coronary occlusions and myocardial infarction, and those with old or recent myocardial infarction.

Table I records the pathologic and electrocardiographic findings in the former group. None of these cases showed any gross pathologic lesions in the ventricular walls or in the septum. However, diffuse myocardial changes or interstitial fibrosis, not visible to the naked eye, occurred quite commonly, both in the ventricular walls and in the septum. A deep or abnormal Q-wave did not appear in any of the electrocardiograms in any lead, although inversion of the T-wave occurred nine times.

The second group consisted of nine cases with old or recent myocardial infarction (see Table II). The posterior part of the septum was involved in eight of the nine cases. In seven of these eight cases there were abnormalities of the Q-wave in Lead III. In the remaining case (No. 6), the pathology involved primarily the anterior ventricular wall and septum, and only a small portion of the posterior part of the septum was involved, by extension. Incidentally, this

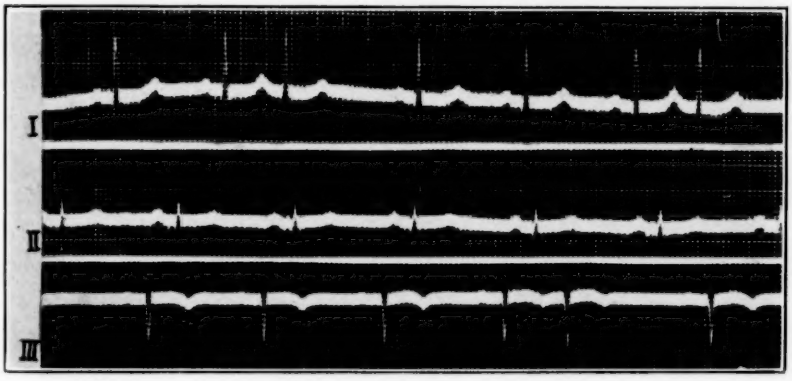

Fig. 2.—Electrocardiogram of the heart in Fig. 1, showing an abnormal Q-wave with inversion of the T-wave and an upward convexity of RT-segment in Lead III.

case showed a deep Q_1 in association with an inverted T_1. Of the seven cases with abnormalities of Q_3, five had definite deep Q_3, one a transient prominent Q_3, and one an initial upward deflection of less than 5 per cent, belonging to the type characterized by Fenichel and Kugell as the indeterminate Q_3.

In three cases, infarction of the posterior ventricular wall was associated with the posterior septal lesions (Fig. 1). In two of these a T_3 inversion was present with a definite deep Q_3. The third case had no inversion of T in any lead and showed an indeterminate type of Q-wave. In one case, although the infarction primarily involved the anterior ventricular wall and septum, it extended back sufficiently to involve the entire lower half of the posterior portion of the septum. This case had a T_1 inversion with the Q_3. In three cases where infarctions occurred in both anterior and posterior portions of the myocardium, as well as in the posterior septal wall, no inversion of the T-wave occurred.

In summarizing, it may be said: That no case with a deep or abnormal Q-wave in Lead III failed to show gross posterior septal pathology; that none

of the cases free from gross lesions in the posterior part of the septum showed a deep Q-wave in Lead III. Seven of the eight cases (87.5 per cent) with posterior septal infarction had a deep or abnormal Q-wave in Lead III. In two of three cases of infarction of the posterior ventricular wall involving the posterior portion of the septum, an inverted T_3 was associated with a deep Q_3; also an inverted T_1 was associated with a deep Q_3 in one instance of anterior ventricular wall infarction extending to the posterior portion of the septum.

CONCLUSIONS

1. A deep Q_3 is a very frequent finding in cases of old or recent myocardial infarctions.

2. A deep Q_3 appears to be correlated with pathology of the posterior part of the septum.

3. The Q_3T_3 combination is suggestive of posterior ventricular infarction associated with posterior septal wall lesions; a Q_3T_1 combination may occur when the posterior part of the septum is involved in the infarction of the anterior portion of the left ventricle and septum.

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TOTAL SULPHUR OF TISSUE IN NORMAL AND ABNORMAL GROWTH (MOUSE CARCINOMA)*

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CONSIDERABLE investigation has been conducted regarding the rôle that sulphydryl compounds play in cellular activity of normal and abnormal growth. It has been known for some time that cystine, the oxidized form of the sulphydryl-containing amino-acid cystine, is one of that limited group of amino acids which is indispensable for normal growth and development. Animals maintained on a cystine-poor diet show lowered vitality, retarded growth and defective hair.† In a previous communication[7] we reported studies and reviewed data showing the important rôle that cystine plays in the formation and development of keratin-containing tissue, especially hair and wool.‡

Whether reduced cystine alone is the primary agent in growth-promoting activity is controversial. Hammett is the chief proponent of this view. From the results of experiments with plant and animal material Hammett and his coworkers[13, 14] concluded that sulphydryl, as the substance occurring naturally in the cell, is the essential and universal stimulus to growth by increase in the number of cells, and that the reaction product, derivatives of sulphydryl are the natural inhibitors of this process so that the stimulation by sulphydryl is normally limited by the inhibitory action of the suboxidized forms of sulphydryl resulting from the normal oxidation of sulphydryl. Reimann and Hammett[15] have reported gross acceleration of wound healing by the simple external application not only of cystine but also of thioglucose and thiocresol.

Hammett's thesis that sulphydryl-containing substances are the essential and universal stimulus for normal growth in plants and animals is of considerable importance from the standpoint of the development and proliferative activity of malignant tumors.

From their studies of the action of sulphydryl on normal cell proliferation Hammett and Reimann formulated the following theory of the origin of cancer: The biologic basis of malignant tumors is the distortion of the sulphydryl equilibrium in the presence in the body of genetically determined lines

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‡Cystine is a unique amino acid since it appears that it cannot be synthesized by the animal organism. Geiling[1] showed that flowers of sulphur did not suffice to prevent loss of weight in rats on a poor cystine diet nor did inorganic sulphate in the study of Daniels and Rich.[2] Compounds closely related chemically to cystine such as dithiopropionic acid and thioglycollic acid[3] or taurine[4] were found inadequate to supplement a cystine deficient diet either for maintenance or growth. Indeed, apparently d-cystine cannot be substituted for the naturally occurring l-cystine in the animal economy.[5] Methionine, however, seems effectively to replace cystine in the diet.[6]

†Animals maintained on a cystine-poor diet soon exhibit lowered vitality, retarded growth[8] and defective formation of tissues. The latter is especially apparent in keratin-containing tissue in which cystine content is relatively high.[9] These symptoms disappear and hair loss is restored when cystine is added to the diet.[10] If the diet is rich in cystine an excessive production of hair[11] and wool[12] is obtained.

of cells in which the heightened nuclear reactivity of hypersensitivity to stimulation of proliferation by sulphydryl characteristic of young, incompletely differentiated cells are retained, without resulting secondarily in increased differentiation. As the diverse architecture of malignant growths is essentially a consequence of the diverse anatomic and physiologic environments in which the proliferation of cells takes place, and as the increase in mitotic activity is in general followed by a decrease in the size of the cells, the rate of tumor growth is directly related to the interpretation of malignancy in terms of sulphydryl calculated from cell size. Sarcomas and carcinomas represent different constitutional (connective tissue or epithelial) types. The difference in hydrogen ion concentration, carbohydrate metabolism, and proteolytic activity of the malignant cell in comparison with the normal cell are but sequelae of the heightened reproductivity of the malignant cell.

In a review and critical analysis of experimental work regarding the etiologic relation of glutathione and sulphydryl to canceration, Huepner[16] concludes: "If one grants sulphydryl substances a contributory part in the development of malignant growth it can be at best only a secondary rôle, remaining in the framework of their normal function."

Among data which Huepner reviewed, which he believed controverted an etiologic relation between sulphydryl-containing substances and malignancy was the findings of a number of investigators that showed that the glutathione content of malignant tumors was not greater than that present in normal tissue.

Besides cysteine, thioneine and glutathione, thiolactic acid, thioglycollic acid and thioprotein may also play a rôle in promoting tissue growth and may all derive their sulphur from cystine in the diet.

In the studies here reported of the relation of sulphydryl compounds to normal and abnormal growth we studied the total sulphur content of the skin of rabbits; and of different organs of mice maintained on normal and on cystine-poor diets; and noted the effect of such diet on the rate of growth of cancer in mice. An attempt was made to correlate the rate of growth with the sulphur content of the tumors. Total sulphur determination* was selected in preference to the estimation of individual naturally occurring sulphydryl compounds. Determination of total sulphur is more accurate and affords a composite picture of all the sulphydryl compounds which may be operative in stimulating growth. Our purpose was not concerned with the relative effectiveness of reduced or oxidized forms of sulphydryl substances but to correlate total sulphur content of tissue in normal and abnormal growth (carcinoma in mice).

The skins of rabbits were employed in our study of the total sulphur content of normal growing tissue. Skin is ideal for such study; it is a rapidly growing tissue; it is relatively high in sulphur;[9] it can be obtained free of blood and extraneous tissue; in normal growth it increases, proportionally, more rapidly in size than other organs. The skins of litter mates of rabbits

*Sulphur was determined according to the method of Stockholm and Koch.[17]

were analyzed for total sulphur from birth to maturity. A series of such analyses were conducted and repeated on a second litter.

The results of this study shown in Table I are the averages of the two determinations for each age interval. It is to be observed that a definite decrease in total sulphur content occurred from birth to maturity. As the rapidity of growth decreased with the age of the animal the sulphur content likewise decreased.

In Thompson and Voegtlin's[18] study of the glutathione of the organs of the white rat it was observed that glutathione declined with the age of the animal. The decline was proportionately greater than that of the sulphur content of the skin of rabbits as shown in Table I.

TABLE I

RELATION OF NORMAL GROWTH TO SULPUR CONTENT OF RABBIT SKIN

AGE	WEIGHT OF RABBIT GM.	SULPHUR IN SKIN % OF DRY WEIGHT
1 day	76	0.68
2 weeks	180	0.69
1 month	252	0.65
3 months	810	0.54
6 months	1200	0.44
8 months	1500	0.46

In order to determine the effect of a cystine-poor diet on the sulphur content of tissue, a number of white mice were placed on such a diet* for five weeks at which time the sulphur content of muscle, liver, spleen, kidney, and skin of the animals was determined.† As a control study the same organs of white mice maintained on a normal diet were examined for total sulphur content.

The results of this study are shown in Table II. The figures for percentage sulphur represent averages of at least four analyses of pooled organs from

TABLE II

SULPHUR ANALYSIS OF ORGANS OF NORMAL MICE AND MICE ON CYSTINE-POOR DIET*

TISSUE	SULPHUR % OF DRY WEIGHT	
	NORMAL MICE	AFTER 5 WEEKS ON CYSTINE-POOR DIET
Muscle	0.49	0.39
Liver	0.59	0.45
Spleen	0.61	0.53
Kidney	0.62	0.44
Skin	0.50	0.41

*Above results seem to indicate a slight but definite loss of body sulphur to meet the demands of metabolism due to withholding cystine from the diet. Mice were on this diet about five weeks.

several mice. It is to be observed in Table II that there is a loss of sulphur in the organs of the animals maintained on a cystine-poor diet. Fifty per

*The diet, a modification of that proposed by Lightbody and Lewis[19] consisted of the following: Dried whole milk 17, gelatin 3, dextrin 75, compressed yeast 1.5, cod liver oil 1.5, and Osborne and Mendel's salt mixture 2.

†Inorganic sulphur was of course included in the determination of total sulphur. The amount of inorganic sulphur in the organs examined is insignificant compared to total organic sulphur.

cent of the animals maintained for five weeks on a cystine-poor diet succumbed. It was, therefore, not practical to prolong the experiment. It is reasonable to assume that more striking results would have been obtained if the animals were maintained longer on the cystine-poor diet. However the results, in contrast to the control study are sufficiently definite to warrant the conclusion that a loss of body sulphur occurs following a cystine-poor diet.

In view of our observations that sulphur content of tissue decreased with the rate of normal growth, and, that loss of sulphur occurred after cystine-poor diet, we conducted the following study to determine the effect of cystine-poor diet on abnormal growth. Fifty white mice were placed on such a diet. At the end of four weeks twenty-two had died. The remaining twenty-eight were inoculated with an actively growing mouse carcinoma and the cystine-poor diet continued. As a control, twenty mice maintained on a normal diet were likewise inoculated. Both groups of mice were examined at weekly intervals for four consecutive weeks following the inoculation. The results of this study are shown in Table III. It is to be observed that the number of "takes" in the group of mice on the experimental diet were considerably less, and that the tumors grew more slowly than in the group on normal diet.

It is well known that under normal conditions of diet and health, some variation occurs in the rate of growth of mouse carcinoma. Generally, however, three weeks after inoculation the majority of mice present a tumor more than one-half the size of the animal. Occasionally, the tumor is smaller and may not attain the usual three-week size until eight weeks or longer. In some animals, too, the usual three-week size is not attained, the tumor stops growing, retrogresses and disappears. In the course of this study such slow growing tumors were observed among the mice in good health and on normal

TABLE III

TUMOR GROWTH IN NORMAL MICE AND IN MICE ON CYSTINE-POOR DIET

NO. MICE INOCULATED	NO. OF "TAKES" AFTER			
	1 WK.	2 WK.	3 WK.	4 WK.
20 (controls)	10	16	18	18
*20 (cystine-poor diet)	0	2	6	7

*Although twenty-eight on this diet were inoculated only twenty were alive at the end of the observation period.

diet. A comparative study of the sulphur content of slow growing, rapid growing tumors, and tumors in various stages of necrosis was made. This group included mice maintained on normal diet with one exception as noted in Table IV. The results as shown in this table are representative of other determinations not listed in the table. These results indicate that the sulphur content of mouse carcinoma correlates the rate of its growth. The more rapid its growth, the greater the sulphur content, the slower the growth, the less the sulphur content. The slowest growing tumor in our series had a sulphur content approximately that of normal muscle (mouse, see Table II) whereas, the sulphur content of the most rapidly growing tumor was more than 50 per

cent higher. The correlation of the sulphur content with the rate of growth applied to animals on normal diet, as well as to the animals on cystine-poor diet.

DISCUSSION

Pertinent to this study is the fact that the organs that continue to grow after full body growth is attained contain a high amount of sulphur, for example, hair, nails, and skin; the more rapidly growing tissues contain more sulphur than other organs. It is not our purpose to endeavor to show that such growth receives its impetus from the sulphur compounds in these tissues; our results indicate, however, a coordination between sulphur content and rate of growth, normal and abnormal. Such coordination has been observed to exist between glutathione content of normal tissue and malignant growths.[20]

TABLE IV

SULPHUR CONTENT OF TRANSPLANTED MOUSE CARCINOMA OF DIFFERENT RATES OF GROWTH

TUMOR	MOISTURE PER CENT	SULPHUR PER CENT DRY WEIGHT
Very slow growing. Not as large in 8 wk. as usual 3 wk. old tumor. *Normal diet*	81.4	0.47
Slow growing. Considerable necrosis. 6 wk. old. *Normal diet*	82.2	0.43
Very slow growing. Mouse on *Cystine-poor diet*	80.3	0.40
Normal growing tumor 3 wk. old. *Normal diet*	80.6	0.61
Normal growing tumor—slight central necrosis 4 wk. old. *Normal diet*	81.4	0.58
Normal growing tumor—considerable necrosis 5 wk. old. *Normal diet*	82.8	0.52
Very rapidly growing tumor. In 10 days had attained size equivalent to normal 3 wk. old tumors. *Normal diet*	80.8	0.74
Very rapidly growing tumor. In 10 days had attained size equivalent to normal 3 wk. old tumors. *Normal diet*	80.5	0.71
Very rapidly growing tumor. Necrotic. *Normal diet*	81.2	0.69

It is to be recalled that tumor growth in animals can be accelerated or retarded under different experimental conditions. Many investigators have held that vitamins A, B, C, and D stimulate the proliferation of transplantable neoplasms. Zondek[21] and his collaborators, and also Cannavo[22] found that subcutaneous injection of anterior hypophyseal hormone retarded the growth of transplanted Ehrlich carcinoma in mice. Frankel and Gereb[23] produced "takes" of Jensen rat sarcoma in mice maintained on a high vitamin diet whereas inoculation was not successful in the control animals on normal diet. Caspari and Ottensooser[24] were able to produce acceleration in the rate of growth of rat carcinoma through the administration of a diet rich in vitamin D.

The state of nutrition of the animal seems to be a factor in growth of mouse carcinoma. Hirata[25] studied the effect of different diets on the rate of growth of Flexner rat carcinoma. It was observed that growth of the tumor was retarded in animals on an inadequate diet, irrespective of the factors concerned in this inadequacy.

In studies published by the British Empire Cancer Campaign, it was found that any foodstuff, whose inclusion in the basal diet improved the general condition of the animals as shown by their appearance, increased life spans and rates of growth, also produced the same effect on the rates of growth of the benign and malignant tissues. Conversely, any food supplement which adversely affected the general condition of the animals decreased the rates of growth of the tumors.

Watson[26] observed an increased carcinogenic response in tar-treated mice when the diet on which they are maintained is supplemented with fresh liver. Such diet did not accelerate the onset of malignancy. There was no difference in the rate of development of malignancy if the administration of liver was discontinued after the first appearance of the benign warts.

The results of Hirokawa's[27] study are pertinent to our results herein reported. He fed different groups of mice rice dough, wheat bread, and "fu" (a kind of cracknel made of gluten). These animals were implanted with mouse carcinoma. The results showed that the percentage of successful tumor inoculations and the rate of tumor growth in mice were distinctly diminished by feeding the animals with "fu" exclusively.

In studies herein reported animals maintained on a low cystine diet were in consequence of such diet in poor nutritional state. As mentioned in the foregoing the mortality rate was high. It is possible that the poor nutritional state of the animals played a rôle in retarding the rate of growth of the carcinoma irrespective of cystine insufficiency. However, since we correlated the rate of growth of transplanted tumors with the sulphur content and since we observed a loss of sulphur in organs of mice maintained on a cystine-poor diet, it is reasonable that cystine insufficiency played some rôle in retarding the rate of growth of the transplanted tumors in the animals maintained on a cystine-poor diet.

The occurrence of slow growing tumors in animals on a normal diet apparently cannot be attributed to cystine insufficiency. This observation, as well as foregoing considerations makes it apparent that other factors are operative in retarding the rate of growth of transplanted tumors. Regardless of these factors it appears from our studies that the rate of growth of transplanted tumors correlates the sulphur content.

SUMMARY AND CONCLUSIONS

Data are reviewed pertaining to the rôle that sulphydryl compounds play in cellular activity of normal and abnormal growth.

Study was made of the total sulphur content of tissue, in normal and in abnormal growth. The skin of rabbits was selected as representing normal

growing tissue. Analyses of total sulphur were made from birth to maturity. It was observed that total sulphur correlated the age of the animal, as rapidity of growth decreased with the age of the animal, the sulphur content likewise decreased.

The effect of cystine-poor diet on the sulphur content of muscle, liver, spleen, kidney, and skin of white mice was studied. There was a loss of sulphur in these organs in comparison with the control animals maintained on a normal diet. It appears from these studies that a loss of body sulphur occurs following a cystine-poor diet.

Total sulphur content of transplanted mouse carcinoma in animals on normal diet and in cystine-poor diet was made. It was observed that the rate of growth of the tumors correlated that sulphur content, the slower the growth the less the sulphur content, the more rapid the growth the greater the sulphur content. Data are reviewed pertaining to factors operative in retarding the rate of growth of transplanted tumors. It appears that regardless of factors involved in retarding the rate of growth of the tumors in the animals studied, the sulphur content correlated the rate of growth.

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2101 PINE STREET

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THE ETIOLOGIC RELATIONSHIP OF AMIDOPYRINE TO AGRANULOCYTOSIS*

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THE rôle that amidopyrine plays in agranulocytosis is not altogether clear. Since 1931, when Kracke[1] suggested the possible relation between this disease and the ingestion of coal tar derivatives, many reports have appeared in this country, Scandinavia, and Holland, viewing the therapeutic use of amidopyrine with disfavor. Foremost in this regard have been those of Watkins,[2] Roberts and Krache,[3] Kracke and Parker,[4] Madison and Squier,[5] Randall,[6] Hoffman, Butt, and Hickey,[7] Holten, Nielsen and Transbol,[8] Seeman,[9] and Fitz-Hugh,[10] among others, each of whom presents cases giving a history of the taking of amidopyrine or related drugs. However, Jackson[11] doubts the significance of these drugs. In his study of twenty-seven cases of agranulocytosis only seven could be attributed, though with question, to the ingestion of amidopyrine.

At the present time there is no convincing experimental evidence that amidopyrine can produce agranulocytosis in laboratory animals, which fact does not necessarily mean that agranulocytosis in man may not be produced by this drug. Madison and Squier[5] reported that they had produced agranulocytosis in a single rabbit but failed in eighteen others, and this solitary positive result has not been repeated by the many others who have given amidopyrine to animals. Incomplete oxidation of the drugs containing the benzene ring (ace-

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tanilid, phenacetine, amidopyrine and arsephenamine) leads, according to Kracke,[12] to the formation of catechol, para-quinone, and quinone, which, he suggests, may be the responsible agents. In a personal communication Kracke and Parker[13] informed me that they have obtained agranulocytosis in rabbits injected with quinone. (I have failed to obtain similar results in twenty rabbits that were injected daily for three weeks by the subcutaneous route with quinone in doses of 0.002 to 0.100 gm.) Miller[14] after administering orally to sixteen dogs 0.3 gm. per kilo of amidopyrine for four weeks found no marked decrease in the number of granulocytes in the peripheral blood. Suggestive, however, is the fact that histologic study of the bone marrow of the femur revealed a decrease or almost complete absence of granulocytes.

In an effort to investigate the effect of amidopyrine on the white and differential blood count I undertook a study of some 120 animals, comprising the guinea pig, the rabbit, and the monkey, over a period of eight months. Amidopyrine (Metz) was given orally to one group in doses from a fraction of a grain to three drams, and blood counts and differentials were taken daily. The symptoms were stupor, diarrhea, and loss of weight. Many of the animals died of pneumonia, some from amidopyrine poisoning, but at no time was agranulocytosis observed. This work was repeated with subcutaneous, intraperitoneal, and intravenous injections, but no change in the blood picture occurred. With another group an attempt was made to sensitize some of the animals treated, and these were given a one-month rest period, after which the medication was again given, still without effect. Incidentally, a pregnant guinea pig was injected with huge doses of the drug for one and a half months, but the newborn guinea pig and the mother both had normal blood pictures.

The next problem contended with was: Do severe anemia, damage to the bone marrow, starvation, chronic infection and toxemia, or cachexia, constitute the soil upon which agranulocytosis develops when amidopyrine is administered? The first group of animals in this series was bled daily until the red blood count fell between 1,500,000 to 2,000,000 and the red cells in the smear showed anisocytosis, poikilocytosis, and polychromasia. Despite this preparation the white blood count failed to fall upon prolonged administration of the drug. In the second group, small quantities of benzene in olive oil were injected subcutaneously until a severe neutropenia and anemia were produced. In most of the animals the white blood count fell to below 1,500. To one-half of this group amidopyrine was given orally and these animals showed, peculiarly enough, a more rapid rise in the white blood count and polymorphonuclear differential picture than in the other animals not given the drug. The animals of the third group were starved for five days and the drug administered, but without avail. In the fourth group animals were injected subcutaneously with broth cultures of *B. subtilis, Streptococcus viridans,* and *Salmonella suipestifer,* and one month later, with the infection still persisting, they were given heavy doses of amidopyrine orally during three weeks. The white blood counts were elevated during the period of amidopyrine treatment, and the differentials showed a predominance of polymorphonuclear leucocytes.

SUMMARY

Administration of amidopyrine to guinea pigs, rabbits, and monkeys, both with and without preliminary anemia or bone marrow injury, failed to produce any appreciable granulocytopenia.

I am indebted to Mr. Maurice Kadin of Chicago and Miss Nettie Dash of Philadelphia for their technical assistance.

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IMMUNITY PHENOMENA IN TUBERCULOSIS*

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THE study of resistance to tuberculous infection embraces numerous biologic interactions. We must consider the cellular factors, the effect of sensitization, intercellular and intracellular digestion, humoral and fixed antibodies, endocrine and vitamin and chemical equilibriums. These factors become more difficult to interpret when pathologic complications, various constitutional differences, disturbances of the autonomic system, metabolic and emotional factors, and environmental influences and differences in the virus are added to the general changes that occur in patients.

The pathologic anatomists have demonstrated the peculiar liability of the slender individual, and clinicians have demonstrated the same fact in clinical studies and analytic analyses. This natural resistance is, according to Petersen,[1] not only genotypic, but may be influenced by such factors as menstruation, diet, season, and psychic status; but constitution, genotypic and paratypic, must be kept in mind as a determining factor in an infectious disease such as tuberculosis.

The human organism in attempting to overcome infection, as in tuberculous infection under discussion, attempts to overcome it in one of two ways, either by resorption or encapsulation. It is obvious that inhibition of digestion and suppression of inflammatory response is of great importance for the improvement of the patient with tuberculosis.

Together with Petersen, I[2] investigated some of the interrelations of resistance and the chemical status of a group of tuberculous patients (all men, 83 in number) in various stages of clinical activity. This investigation among other things included a study of the constitution, calcium-potassium ratio, cholesterol, tuberculin, and reaction to epinephrine. We wish to review briefly the latter findings in relationship to tuberculosis.

Constitution.—As far as the patient with pulmonary tuberculosis is concerned, increased inflammatory reactivity is usually associated with increased clinical activity. When the constitutional reactivity is lessened, the clinical course is relatively benign. Among the various lines of approach, particularly in the form of therapy, emphasis is directed toward specific immunity, with the attack directed toward the virus; chemotherapy with the emphasis against the destruction of the specific virus without injury to the cell; and last, the beneficial reactions of the tissue cells as a result of stimulation, with the accompanying suppression of the unfavorable ones.

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The result of clinical and laboratory study has given us an opportunity of classifying patients into the sympathicotonic and vagotonic groups. It is obvious that the autonomic nervous system as a general part of the vegetative balance is involved. Epinephrine makes the capillary endothelium less permeable and is the representative agent for producing sympathetic effects. The "sympathicotonic" person has more permeable capillaries and the blood pressure may be low. The rest stage may be looked upon as the state of tissues with contracted vessels and less permeable capillaries. However, in some patients the injection of epinephrine hydrochloride lowers the blood pressure and increases the permeability. In the "vagotonic" person the blood pressure is higher, the metabolic rate is lowered, and the capillaries are less permeable. Stimulation of the so-called parasympathetic nerves may result in dilatation of the vessels and increased permeability which may mean tissue activity. In terms of clinical medicine the function of the capillaries may be expressed thus: Relatively impermeable capillaries are usually found associated with relatively chronic tuberculosis, and greater permeability with active tuberculosis.

The conclusion would be that increase in capillary permeability, however produced, unfavorably influences a tuberculous process.

Calcium-Potassium Ratio.—In a small series of cases studied by Voorhoeve,[3] he maintains that he noticed no striking difference in the calcium balance in tuberculous and nontuberculous subjects. Bonanno[4] in his study of 60 cases of pulmonary tuberculosis in various states reports no appreciable change in the calcium potassium content of the blood commensurate with the severity of the disease. A similar observation was made by Varela[5] who studied the total calcium of the serum and the fraction of ionized calcium in tuberculous patients and found that they remain within normal limits. Brockbank[6] observed that when his patients were graded according to severity, on the average the calcium was decreased in quantity in the serum when the disease was acute, and that it was increased when the disease was healed, with proportionate results in the intermediate stages. The difference amounted to about 20 per cent. The serum calcium was not diminished in patients who are coughing up blood, as compared with patients in a similar stage of the disease but without that symptom.

In an analysis of our 83 tuberculous patients we find that when this group is arranged according to clinical classification as minimal, moderately advanced, and far advanced, it is noticed that the minimal group has the highest value of potassium-calcium ratio and the more advanced lower values, but there is no progression or regularity. If the patients are classified according to the weight curve, there is a reduction of the calcium from the gaining to the losing group. When a similar study was made of these patients on the basis of prognosis, those who were gaining had the highest calcium levels. The significance of the calcium metabolism in our patients studied indicates that those who were gaining maintained a high calcium level as part of their picture of resistance. This occurs without loss from the body and the patients are said to be in "calcium balance." The group of patients who were losing were in a negative calcium balance, although the calcium level was high as it represents the calcium leaving the tissue and passing out from the body.

As far as potassium is concerned, we were unable to ascertain any clear-cut influence of the potassium levels on any clinical features studied. The potassium-calcium ratio has less influence on the tuberculous process than has the actual amount of calcium.

Cholesterol.—Clinical study has shown that tuberculous patients who have a high cholesterol content tend toward improvement, and conversely as the cholesterol content decreases the prognosis is grave. Gavrila and Vior,[7] from their study of the cholesterol content of the blood serum from 100 patients, conclude that in the localized and diffuse fibrous forms of pulmonary tuberculosis there occurs a hypercholesteremia, particularly when the lesions are inactive and are not undergoing evolution. Hypocholesteremia is found in the diffuse ulcero-caseous tuberculosis when the lesions are active.

The cholesterol study made on our tuberculous patients substantiates the findings of Gavrila and Vior in that the patients with good prognosis and inactive lesions had a higher cholesterol ratio than the patients with poor prognosis and active lesions. We have also noted that exudative pleurisies do not seem to influence materially the cholesterol content. In fact the highest cholesterol content was noted in some of our patients who had pleurisy.

Experimentally, Shope[8] has demonstrated that cholesterol administered intraperitoneally prolonged the lives of tuberculous guinea pigs when the infection was of an acute type produced by the injection of small doses of virulent human tuberculous organisms. When the infection was of a chronic type produced by the injection of small doses of human tuberculosis of a low virulence, the intraperitoneal injection of cholesterol did not prolong the lives of the guinea pigs.

Hinze[9] examined 69 patients with pulmonary tuberculosis and concludes that the greater the cholesterol content of the blood the greater the resistance of the patient to tuberculosis.

Palacio[10] attempts to prognosticate the outcome of pulmonary tuberculosis by cholesterol determinations and concludes that the quantity of cholesterol in the blood constitutes an index to the general condition of the patient.

There are several factors which modify the cholesterol content of the blood in tuberculous patients, namely, activity or inactivity of the lesions; state of immunity of the organisms; the pathologic anatomic form of the disease.

Gavrila and Vior are of the opinion that hypercholesteremia indicates a state of a well-developed immunity and inactive pulmonary lesions, and is, therefore, of good prognostic import, while hypocholesteremia denotes a feeble state of immunity and acute lesions, and is, therefore, of bad prognostic import.

Tuberculin Reaction.—When making tuberculin skin tests, we must bear in mind the reactivity of the skin and the speed of the reaction before interpreting the results in terms of immunity. Thus the size of the reaction at the site of the injection of tuberculin, as measured after twenty-four hours, varied in our series from 0 to 28 mm. There is no difference in the clinical prognosis of our patients as determined by the tuberculin reaction. It was noted that those patients who gave a small skin reaction usually were patients with min-

imal clinical manifestation. The group with clinical manifestations of far-advanced tuberculosis gave the larger reactions.

The persistency of the tuberculin reaction was not in direct relation to the deaths. We observed that the cases in the far-advanced group showed a persistent and marked flare, and the gain in weight was associated with the largest reactions.

Ossoinig[11] observed at different times a peculiar increase and decrease of the tuberculin reaction occurring in the majority of the patients tested. He believes that climatic influences may play a rôle, but has not definitely been able to correlate them.

Reaction to Epinephrine.—We have divided the intracutaneous reaction to epinephrine from the observations made of the wheal and the flare. The average size of the wheal at the site of injection in our "normal" series was 19.2 mm. and in the tuberculous group it averaged 15.2 mm. More deaths occurred in our patients with small wheals, while in the group with large wheals, the prognosis seemed better, and more of the cases were at least stationary. The group with the most favorable prognosis seemed to be that in which the wheal was intermediate. Our study further indicates that the flare of the reaction to epinephrine and capillary permeability are somewhat greater in the group with large wheals; and a similar manifestation holds true for the reaction to tuberculin.

The average flare following the intracutaneous injection of epinephrine of "normal" men had a radius of 8.8 mm., while in the tuberculous patients it had a radius of 6.8 mm. The deaths in our series occurred in the group with the smaller flares. There appeared to be no significant relation of the size of the flare to the clinical classification, the prognosis, or the weight curve.

DISCUSSION

In order to correlate a number of biologic reactions that are observed in diseased conditions, it is necessary to know similar changes occurring in "normal" individuals. These observations are to be correlated with clinical symptoms and with resistance to an infectious disease like tuberculosis. It is important, therefore, to determine the alterations in the biologic tests employed in order to estimate increased or decreased resistance to tuberculosis.

In the previous report we[12] have shown that resistance to tuberculosis means complete walling off from general tissue activity, that is impermeability: anatomically, in the sense of a complete connective tissue encapsulation; and functionally, in the sense that there shall be lessened exchange of products between the focus and the normal tissues. Conversely, absorption of bacillary and tissue débris may lead to the dissemination of the virus.

Substances that stimulate and dilate capillaries, with incidental increase in permeability, must be potentially injurious. Substances that fatigue the capillary (with delay in its reversibility) must be harmful. Substances that make the capillary less permeable must be essentially beneficient.

When capillary endothelium is stimulated and becomes more permeable, calcium leaves the cell and the tissues become more acid. This is associated

clinically with exudative and progressive tuberculosis, with the increase in parasympathetic tonus (blood pressure lowered, low blood sugar, increased catabolism, etc.). With lessened permeability of the capillary wall the arteriole tonus is high, blood sugar (liver function) normal, tissues bind calcium, and the reaction is toward the alkaline side.

Constitution.—In view of the fact that constitution plays such an important rôle in resistance to infection, a brief review of this subject and its clinical applicability will be given. Von Versucher[13] is of the opinion that constitution is an organically fixed condition. Bauer[14] places emphasis on the importance of constitution in differentiating a group of biologically inferior persons, extreme variants from the normal, in adaptability, in vitality, and in resistance. For practical purposes for study, persons have been grouped as (1) normal (normotype), (2) slender (asthenic type with small heart), (3) heavy (pyknic type). In a similar fashion the French school associated the slender type with lessened resistance to pulmonary infections, and the heavy type with diabetes, arteriosclerosis and rheumatism. The following classification of the slender and broad types is taken from Petersen, "Constitution and Disease."[1]

THE SLENDER TYPE	THE BROAD TYPE
(Leptosome, longitudinal, linear, dolichormorphic; asthenia as an extreme variant)	(Plethoric, apoplectic, pyknic, gouty, "arthritismus," the round type, brachymorphic)
The individual is long	The individual is broad
Extremities are long	Extremities are short
The face is narrow, oval; the nasal bridge is narrow	The face is broad, round; the nasal bridge is broad
The neck is long	The neck is short
The chest is long and flat, the costovertebral angle is small	The chest is barrel-shaped, the costovertebral angle is larger, the intercostal space is narrower
The diaphragm is low	The diaphragm is high
Dolichocephalic	Brachycephalic
Poor connective tissue reactivity	Pronounced connective tissue reactivity (fibrous)
Bony framework is delicate	Bony framework is strong
Skin is thin	Skin is heavy and coarse
Hair is fine, usually well preserved	Hair is coarse and abundant, frequently lost early
The tissue storage is deficient (few reserves)	The tissue storage is pronounced (reserves good, especially fat, glycogen, etc.)
The heart is small (hypertrophy poor)	The heart is large (hypertrophy good)
The arterial system is narrow	The arterial system is large
The superficial capillaries not marked	The superficial capillaries dilated
The viscera are mobile	The viscera are well fixed
Visceroptosis, varices, intussusception, prolapses and hernias common	Rare
Bodily circumference small	Bodily circumference large
Smooth muscles hypotonic	Smooth muscles hypertonic

Sluggish general reactions except nervous and vasomotor systems	Hyperirritable in childhood; sluggish in later life
Thyroid activity may be increased	Thyroid activity lessened
Psychic classifications, schizothymic	Psychic manifestations, cyclothymic
Larynx large, voice barytone	Larynx small, voice tenor
Far sighted	Near sighted
Mechanical adaptation to overcome distance and time	Mechanical adaptation to overcome mass
Disease disposition, tuberculosis	Disease disposition, early exudative diathesis, late wear and tear diseases, metabolic diseases (gout, diabetes, concretions, sensitizations, etc.)
Moderate lymphatic reaction	Pronounced lymphatic reaction

When we attempt to interpret the results of our investigations on the basis of the above constitutional types, we notice that in the group of slender persons there is a low calcium-potassium ratio, an increased vascular reaction to epinephrine, lower cholesterol, and a lesser reaction of the skin to epinephrine. The slender person, in contrast to the heavy person, has an increased basal metabolic rate, a slightly lower CO_2 combining power of the serum, higher resistance of the skin to electric current and a longer Kromayer light reaction time.

Aside from the constitutional types of persons, the age of the individual may probably be considered in reference to tuberculosis. Lydtin[15] examined in approximately 200 patients the relation of the age of the individual to the form of tuberculosis. The percentage of the proliferating, cirrhotic and exudative forms is the same in every age. The development of these forms is different. In younger persons the tendency goes from a proliferating to an exudative form, while the latter in older people has temporary ameliorations of the cirrhosis and cavity types.

Another phase in the constitutional types in reference to resistance is the question of the height of the individual. Frassetto[16] found that the average height of recent levies for the army is 2.5 cm. greater than that of the levies of 1859-1863, not only in healthy men but also in tuberculous patients. The height of patients with pulmonary tuberculosis exceeded by 2.5 cm. that of the healthy subjects, not only in recent but also in old levies. The chest girth in pulmonary patients is diminished by 2.6 cm. in the recent as compared with old levies, while in the healthy subjects, the reduction amounts to only 1.5 cm. In the old levies the chest girth of the tuberculous differed from that of the healthy subjects by 0.9 cm., and in the recent by 2 cm. The average weight of the tuberculous (aged twenty to fifty) in the recent levies is 12.5 kg. less than the weight corresponding to the average height of the tuberculous subjects, which was 167.5 cm.

Seasonal changes are known to influence the course of tuberculosis. Grönberg's[17] studies on 2,180 patients with pulmonary tuberculosis in Finland, comprising about 42,000 weighings and covering a period of seven years, show that the curve of the average monthly increase in body weight was practically

the same from January to April (245 to 262 gm.) dropping somewhat in May (232 gm.), rising rather sharply during the summer months to reach its peak in September (385 gm.), then sinking to the minimum in December (216 gm.). There was marked similarity between the weight curves and the curves of the average monthly temperature of the air and hours of sunshine. These observations on the relation between warmth and sunshine and body weight agree with results from sanitariums for the tuberculous in widely different parts of the world.

Calcium.—Zondek[18] calls attention to the fact that two balances are of greatest physiologic importance, the H ion concentration, and the potassium-calcium ratio. With cellular activity, the calcium concentration of the cell membrane is diminished. Calcium leaves the cell, and there is a relative increase in potassium in the cell. The cell is more permeable and the protein becomes hydrated (Zondek[19]). Thus, cellular activity can be measured by calcium and potassium concentrations. From the stage of activity that of rest is reached with an apparent increase of calcium at the cell surface, relative impermeability and dehydration. The concentration of either calcium or potassium in the serum may mirror the cellular change, but the serum levels do not change the reaction of the cells.

In reference to calcium metabolism it has been shown that it is closely associated with the clinical condition of the patient. We have observed, for example, that more deaths occurred in our patients who have had a low calcium level. Patients with healed tuberculosis have a higher calcium level, and those whose prognoses are good also have a higher calcium content. In contrast to this, we have observed in our study that patients with poor prognoses have a low calcium level. There apparently must exist some correlation between the calcium level and the activity of a tuberculous process, as there is some importance in the calcium in tuberculosis in regard to the reactivity of the tissue and its influence on the disease process. For example, Sternberg[20] found a lower serum calcium in most advanced cases while Schaeffer[21] has found increase in calcium in the lung in chronic tuberculosis. Hoyle[22] has noted some clinical improvement in patients with a higher calcium level; in fact, Hennes[23] noticed that animals exposed to calcium dust or other dust showed less tuberculosis than the control animals which were not exposed to dust. This fact has been applied therapeutically, and Siegel[24] has observed improvement in his patients who were treated by calcium inhalations.

Cholesterol.—It has been shown that cholesterol metabolism is diminished in infectious diseases and particularly with progressive tuberculosis. Shope has observed that as the cholesterol level is increased the prolongation of life in experimentally infected guinea pigs was noted. In fact, the relationship of cholesterol and other fats in the treatment of tuberculosis is an old empiric observation and is one of the factors that is still used today as a form of therapy in tuberculosis. We have observed that as a rule, in our investigations, low cholesterol in tuberculosis is associated with poor prognosis; a similar observation was noted by Reiter.[25]

Skin Reactions.—In reference to the reactivity of the skin in tuberculosis we have used the cantharides plaster applied to the skin.[26] This is a chemical irritant and acts primarily on the cell membranes. Specific sensitization is not employed in this test but the analogy to the tuberculin reaction is made here because of the skin irritation produced when a specific and nonspecific skin test is employed.

During the febrile stage there exists a peripheral vasoconstriction. The cantharides can act on the cell, but little or no exudate forms. There is direct injury to the cells. Applied to tuberculin and the tuberculin reaction, it means

TABLE I

COMPARISON OF CALCIUM, POTASSIUM, CHOLESTEROL, EPINEPHRINE, AND TUBERCULIN LEVELS IN PULMONARY TUBERCULOSIS

		MINIMAL	MODERATELY ADVANCED	FAR ADVANCED
Calcium		9.9	10.5	10.3
Normal	9.7			
Potassium		19.6	18.3	19.0
Normal	20.3			
Calcium Potassium Ratio		2.0	1.76	1.88
Normal	2.1			
Cholesterol		235.0	200.0	157.0
Normal	140-170			
Epinephrine				
Wheal		Medium wheal	Small wheal	Large wheal
Flare		Small flare	Medium flare	Large flare
Tuberculin				
End of twenty-four hours		Small wheal	Medium wheal	Large wheal
Persistent reaction		Small reaction	Medium reaction	Large reaction

that during the febrile stage of an intercurrent disease the tuberculin reaction is inhibited, because of the change of the status of the skin. During the terminal stage of tuberculosis this factor is also operative. During the stage when there is a peripheral vascular dilatation, when the cantharides plaster is applied, a strong local skin reaction results. Applied to the tuberculin reaction this corresponds to the period of maximum response when sensitization takes place at a time during which peripheral vasodilatation is compensating for the increased metabolism going on in the body. The autonomic skin function is practically normal as far as the lability of the skin is concerned. Then as the pathologic lesion in the lung, for example, becomes quiescent and the patient goes on to recovery, the cell membranes as in the skin are no longer irritated to the same degree by the same amount of irritant. In other words, the cell membranes have become less permeable. These reactions are wholly nonspecific effects which influence the reactivity of the skin in general, but they are of importance for the problem of tuberculosis, because the vegetative status of the skin and the vasomotor activity are underlying factors that determine the type of reaction to all toxic agents.

It is obvious, therefore, that in the intricate biologic picture of inflammation presented by tuberculosis, innumerable possibilities exist. We should emphasize that in clinical tuberculosis it is the vascular mechanism that must always be in the foreground of consideration.

CONCLUSIONS

1. We have made biochemical studies on 83 patients with pulmonary tuberculosis (minimal to far advanced) and have here discussed briefly the subject of constitution, calcium, potassium, cholesterol, epinephrine skin tests, and tuberculin in relation to resistance to tuberculosis.

2. The results indicate that substances that stimulate and dilate capillaries, with incidental increase in permeability, must be potentially injurious to the host. Substances that make the capillary less permeable must be essentially beneficial to the organism.

3. As the clinical status in tuberculosis progresses (exudative and progressive tuberculosis), the capillary endothelium is stimulated and becomes more permeable, calcium leaves the cells, and the tissues become more acid (parasympathetic tonus, low blood sugar, increased catabolism, etc.).

4. When the organism is in a state of lessened permeability of the capillaries (sympathetic tonus, blood sugar normal, tissues bind calcium), the reaction is toward the alkaline side and the permeability of the capillaries is lessened; that is, the organism is in the state of sympathetic tonus, the blood sugar is normal and the tissues bind calcium.

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THE EFFECT OF HIGH PROTEIN DIETS ON THE KIDNEY FUNCTION IN DOGS*

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THE object of the investigation discussed in this paper was to determine whether or not the renal and arterial disease described by L. H. Newburgh† in rabbits after feeding high protein diets could be produced in adult dogs. Dogs were chosen because their naturally selected diet more nearly approaches the human diet than that of any other laboratory animal.

METHOD OF STUDY

Five disease-free female dogs were operated upon under ether anesthesia and a small block of tissue for microscopic examination was removed from the upper pole of the left kidney. After about four weeks the kidney function, as determined by qualitative analysis of the urine for albumin, blood, casts, and cells, the total nitrogen of the urine by the Kjeldahl method, the phenolsulphonphthalein dye test determined in a Klett biologic colorimeter, and the nonprotein nitrogen of the blood, had returned to normal. The animals were then fed quantitative diets which contained 5 gm. of protein per kilogram body weight and afforded 50 calories per kilogram. This caloric intake was necessary because the dogs were permitted exercise except during the short intervals when they were in the metabolic cages. The diets were made up of ground meat, composed of seven-eighths round steak, one-sixteenth each of heart muscle and liver, and lard. To this diet was added 0.01 gm. of calcium as calcium carbonate and 0.30 of a minim of Meade's viosterol in oil 250-D per kilogram. Sufficient sodium chloride was added to season. This diet fulfills all the nutritional requirements and, of course, was readily taken by the dogs. The control was fed the stock laboratory diet. The feeding was continued for five months, at the end of which time the kidney function was again determined, the dogs reoperated upon, and tissue from the right kidney of each dog was removed for microscopic examination.

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†Newburgh, L. H., and Clarkson, Sarah: Arch. Int. Med. **32:** 850, 1923.

RESULTS

The figures in Table I indicate the kidney function at the beginning and at the end of the experiment.

Microscopic examination of the kidney tissue removed before the experiment and on the last day of the five-month feeding period revealed normal kidney parenchyma and vascular system.

TABLE I

			AT BEGINNING OF EXPERIMENT				AFTER HAVING BEEN ON THE HIGH PROTEIN DIETS FOR FIVE MONTHS			
DOG	DIET	WEIGHT (KG.)	QUALITATIVE ANALYSIS OF URINE FOR ALBUMIN, CELLS, BLOOD, CASTS	TOTAL NITROGEN IN URINE	NONPROTEIN NITROGEN OF BLOOD	PHENOLSULPHONPHTHALEIN EXCRETION TEST	QUALITATIVE ANALYSIS OF URINE FOR ALBUMIN, CELLS, BLOOD, CASTS	TOTAL NITROGEN IN URINE	NONPROTEIN NITROGEN OF BLOOD	PHENOLSULPHONPHTHALEIN EXCRETION TEST
I	CHO – P 32.4 F 21.9 Cal 325.0	6.5	Normal	5.2	0.028	65%	Normal	4.8	0.028	68%
II	CHO – P 48.2 F 33.3 Cal 493.0	9.6	Normal	6.8	0.036	61%	Normal	6.9	0.040	62%
III	CHO – P 56.2 F 34.0 Cal 530.0	10.8	Normal	9.0	0.035	62%	Normal	8.2	0.036	93%
IV	CHO – P 50.2 F 33.7 Cal 504.0	10.2	Normal	7.4	0.035	62%	Normal	7.8	0.028	90%
V (Control)	Stock diet	14.4	Normal	--	0.037	94%	Normal	---	0.029	84%

Two of the dogs went through pregnancy and gave birth to normal full-term pups without any evidence of kidney damage or toxemia. All of the animals were in excellent physical condition at the end of the experiment.

CONCLUSION

Diets containing 5 gm. of protein per kilogram body weight and fulfilling all nutritional requirements when fed to adult dogs for five months produced no kidney damage that could be demonstrated by standard renal function tests or by microscopic examination. Attention may be drawn to the fact that five grams of protein per kilogram is four to five times higher than the average American adult consumption, and that five months in the life of a dog compares with two years in the human being.

BLASTOMYCOSIS IN AN INFANT SIX MONTHS OLD*

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THE reason I am reporting this case is because of the age of the patient, and the rarity of the disease in this part of the country. This case was referred to our laboratories by a local physician who, after exhausting his medical and therapeutic knowledge, decided that laboratory studies should be undertaken in the interest of his patient.

According to Pussey's *The Principles and Practice of Dermatology,* blastomycosis is a specific infectious disease produced by a yeast fungus and characterized, as it occurs in the skin, by the formation of elevated, warty, suppurating lesions with abruptly sloping purplish red borders which are studded with pinpoint-sized deep-seated epidermal abscesses, from the pus in which pure cultures of the organism can usually be obtained.

The recognition of blastomycotic dermatitis as a pathologic entity dates from the meeting of the American Dermatological Association, in May, 1894, when Gilchrist demonstrated sections containing budding organisms which had been taken from a scrofuloderma-like lesion.

In November, 1894, Busse reported a fatal case of pyemia with subcutaneous abscesses and cutaneous lesions in which the pathogenic agent was yeast.

July 7, 1894, Buschke (Greifswald Medical Society) referred to a case with skin lesions in which he found coccidial bodies. In Chicago the disease was studied by Rickets, Hyde, Montgomery and Ormsby. The name of blastomycotic dermatitis was applied by Gilchrist. The name blastomycosis suggested by Hyde is perhaps better.

Histology.—The histologic features of this case were as follows: Proliferation of epithelial cells of the rete, formation of miliary abscesses within the epidermis and acute inflammatory changes in the corium. The cells of the rete were larger and swollen, and within the cells there were polymorphonuclear leucocytes, cellular detritus, some giant cells and a varying number of specific organisms.

The physician requested us to make a study of the scrapings from the epidermal abscess, from a microscopic and bacteriologic standpoint. We made several cultures and smears from the material in our effort to classify the mould and identify any organism which we might find. On microscopic study, we concluded that the causative organism was the *Crytococcus gilchristi,* found in the purulent discharge as a round, doubly contoured budding yeastlike cell, 10 to 12 microns. In culture we saw the formation of a mycelium resembling that of an oidium.

*From the Hamilton Laboratories.
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The media used was glucose agar, and after three days a white fluffy growth with aerial hyphae was observed. Stained with the thionin stain, mycelium was seen with sporelike bodies embedded in it.

The child had this condition for a period of three weeks, and after treatment with potassium iodide and x-ray applications, the child recuperated fully after six months' treatment.

This case was demonstrated at the Dermatological Clinic at Temple University, Philadelphia, and it was the first case which they had observed in a child so young. The diagnosis was confirmed at Temple University.

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THE EFFECT OF FORMALDEHYDE ON THE GROWTH OF TUBERCLE BACILLI*

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THE sterilizing action of formaldehyde on nonspore-forming bacteria is well known, and this method is commonly used for sterilization of instruments or other equipment that may be damaged by other means.

Previously I had noted that the growth of certain pathogenic bacteria such as staphylococci and colon bacilli was inhibited or prevented after inoculation when the Petri dishes were placed in closed cans containing formaldehyde.

It seemed worth while to determine the relative resistance of tubercle bacilli to formaldehyde as compared to contaminating bacteria with the idea that such treatment might prove to be a satisfactory substitute for the acid or alkaline treatment of sputums or other suspected tuberculous material before inoculation of culture medium when such materials contained other bacteria.

Sputums positive for tubercle bacilli were selected for the experiment and the cultural observations were made on an egg yolk agar medium that I† have

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previously described. I have found that when the egg yolk is added to the melted agar according to the technic described in the above reference, a very small percentage of contaminations result on the uninoculated plates or slants. This has been confirmed by Woolsey[2] and Feldman,[3] the latter of whom states that it was unusual for a tube to be lost on account of contamination when the egg yolks were used. I believe that the flaming of the shell through a Bunsen burner before opening destroys any sporogenous bacteria that may be dormant between the outer shell and inner membrane, whereas these organisms are not destroyed when the eggs are treated with phenol and alcohol as is commonly done in the preparation of Löwenstein's and other similar mediums. A pledget of cotton saturated with formaldehyde was placed in the bottom of the closed containers of two quart capacity which were used for exposure of the inoculated specimens.

Two parallel series were tested for the effect on the growth of the tubercle bacilli and other bacteria after exposure of the sputum inoculum to formaldehyde for periods of two and twenty-four hours, after which the inoculated plates were removed from the formaldehyde cans, incubated, and readings made at intervals for thirty days.

In the first series the sputum, positive for tubercle bacilli, was treated for one-half hour with 3 per cent sodium hydroxide, and three egg yolk agar plates then were inoculated heavily with an equal amount of centrifuged sediment. The control plate that was incubated without exposure to formaldehyde gave the first macroscopic appearance of tubercle bacillus colonies five days after inoculation, and by the ninth day there was a heavy growth. The plates subjected to formaldehyde for two hours at room temperature gave the first macroscopic appearance of growth on the ninth day, and reached its maximum growth on the eighteenth day, at which time there was about 50 per cent inhibition as compared to the control plate. The third plate was left in the formaldehyde container for twenty-four hours at room temperature, after which it was removed and placed in the incubator. There was no growth of tubercle bacilli or other bacteria during the thirty-day period of observation.

The second series consisted of inoculation of three plates with the same positive sputum which was not treated by sodium hydroxide. The control plate at the end of twenty-four hours was completely covered with a confluent growth of a mixture of gram-positive cocci and bacilli. The second plate which had been placed in the formaldehyde can for two hours before incubation gave a similar growth of flora as the control plate at the end of twenty-four hours, but there was approximately 50 per cent inhibition in the growth of the same mixture of gram-positive cocci and bacilli. The third plate which had been left in the formaldehyde can for twenty-four hours before incubation did not yield any tubercle bacilli or other bacteria during the thirty-day period of observation. This experiment was repeated with a second positive sputum with similar results. On the plates which had been subjected to the formaldehyde for two hours, there was a growth of tubercle bacilli equivalent to about 25 per cent that of the control of the first series treated by sodium hydroxide, and there was a comparative delay of about one week in the appearance of colonies.

DISCUSSION

It is apparent from these observations that tubercle bacilli are relatively more susceptible to formaldehyde as compared to other bacteria than when treated with acid or alkalies in the strength commonly used for destruction of secondary bacteria in the cultivation of tubercle bacilli. Obviously the above observations indicate that formaldehyde is not a satisfactory substitute for the acid or alkali treatment of specimens before inoculation of culture mediums.

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CONCOMITANT COMPLETE HEART-BLOCK AND BUNDLE-BRANCH BLOCK*

A Theoretical Discussion

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ADMITTEDLY there is every reason to believe that complete auriculoventricular dissociation and bundle-branch block may coexist. However, a concomitant diagnosis of these two lesions cannot be made in every instance that the electrocardiogram seems to indicate the association. This theoretical discussion appears advisable in view of the frequency with which such a combined diagnosis is made unreservedly.[1-8]

Even though experimental bundle-branch block produces such a characteristic electrocardiogram that there can be no reasonable doubt that a similar human electrocardiogram must indicate bundle-branch involvement, there are a few conditions which must be excluded. Paroxysmal ventricular tachycardia produces complexes similar in form to bundle-branch block. Likewise paroxysmal auricular tachycardia with the impulse pursuing an aberrant course in the ventricle might resemble auricular tachycardia with a bundle block. Finally the idioventricular rhythm in complete A-V dissociation *due* to a focus of impulse formation *below the main stem* of the bundle of His would resemble that of branch block. If, in complete heart-block, an idioventricular impulse originates

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in the main stem but is blocked in the bundle or if the focus is *below the* main stem, the end-result of the spread of the contraction over the ventricles would be the same and produce similar electrocardiographic findings.

The combined diagnosis of complete heart-block and bundle-branch block is not warranted without excluding a rhythm originating below the division of the main stem. In complete dissociation of the auricles from the ventricles the idioventricular rhythm usually originates in the main bundle below the site of the injury. Of this the electrocardiogram furnishes proof. The normal ventricular complex is preserved, being the same both before and after the injury. This fact indicates that the impulse arises somewhere between the lesion and the point at which the bundle divides into its right and left branches.[9] Hence, in a slow idioventricular rhythm with bundle-branch type of QRST complexes it is ordinarily assumed that the idioventricular focus is in the main stem and that there is a coincident lesion of one of the branches which accounts for the abnormal complexes. However, aberrant ventricular complexes in complete block must be critically interpreted because portions of the ventricle, other than the His bundle, are capable of initiating an idioventricular rhythm. Lewis[9] states: "In the ventricles, the bundle is certainly endowed with rhythmic power; its continuation in branches of similar elemental constitution in dog and man suggests that these may possess similar properties. It is said that the main divisions readily become rhythmic on heating and we know that when both divisions of the bundle are cut the ventricle continues to respond to impulses generated within itself. The cells of the arborization also possess rhythmicity. . . ." Idioventricular pacemakers, other than those in the main stem, would produce complexes resembling those found in bundle-branch block or even with more diverse and bizarre features. It is apparent, therefore, that the concomitant diagnosis of complete A-V dissociation and bundle-branch block cannot be made unreservedly.

Failure to make a critical differential diagnosis may explain the frequency with which such a concomitant diagnosis has been made.[1-8] Ellis and Weiss[10] included four cases of bundle-branch block among forty-three cases of complete A-V block. In one case the original electrocardiogram showed complete heart-block with left bundle-branch block. A few days later another electrocardiogram resembled the preceding except that right bundle-branch block now existed. They state: "A possible explanation for the development of first left and then right bundle-branch block in successive electrocardiograms is that the origin of the impulse which excited the ventricular contraction was first in one branch of the bundle of His and subsequently shifted to the other branch." In a recent study of 155 cases of bundle-branch block, King[12] reported an associated complete A-V block in thirty-six instances. He noted in such cases a tendency for the bundle block to shift from side to side, and in one case it was thought that the origin of the impulse was not in the main stem below the site of the A-V block but probably first in one ventricle and subsequently in the other. In the light of our discussion above we seriously question whether one would be justified in reporting such combined diagnoses without pathologic confirmation or qualifying reservations.

A definite diagnosis of combined lesions might be made from comparative records. Thus, if a patient with bundle-branch were observed to develop complete A-V dissociation and the bundle-branch complexes persisted, it would be reasonable to assume that two entities were present. Also, if a patient with complete heart-block exhibited a change from supraventricular to bundle-branch complexes, a combined diagnosis would seem justified.

CONCLUSION

We believe that it is theoretically impossible, from a single record, to make a concomitant diagnosis of bundle-branch block and complete heart-block.

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HIGH BLOOD UREA NITROGEN NOT DUE TO CHRONIC NEPHRITIS

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THE impression gained from a perusal of the literature, as well as from modern textbooks of medicine, is that an increase in the nitrogenous waste products in the blood is indicative of chronic glomerular nephritis; this notwithstanding the appearance in recent literature of reports of high blood urea nitrogen not associated with chronic kidney disease.

The importance of recognizing these states clinically and early lies in the fact that many of them are mistaken for coma of renal origin, especially since traces of albumin and some casts are not infrequent urinary findings and furthermore because immediate proper treatment of the nonrenal cases has often proved life-saving.

A glance at Table I will indicate the common findings in the two conditions which serve to differentiate them.

Up to the present time a variety of clinical conditions with which high blood urea or nonprotein nitrogen values are found have been reported:

1. Vomiting from many causes:
   a. Cancer of the stomach[1]
   b. Pylorospasm[2]
   c. Hyperemesis gravidarum[3]
   d. Gastric tetany[4]
   e. Alkalosis in alkaline treatment of peptic ulcer[30]
   f. Experimental pyloric occlusion[5]
   g. Acute intestinal obstruction[10, 11, 12, 13]
   h. Acute general peritonitis[14]
2. Repeated gastric lavage[6]
3. Diarrhea[7, 8]
4. Acute cerebral accidents[9]
5. Reflex anuria[9, 15]
6. Diabetes mellitus[16]
7. Hyperparathyroidism[17]
8. Extensive burns[18]
9. Pancreatic necrosis[19]
10. Hypersensitiveness[20]
11. Infectious diseases[29]

In addition to the above we have had under observation a number of cases with conditions illustrating the phenomenon of high blood urea nitrogen, some not hitherto reported.

CASE 1.—Vomiting associated with obstipation. Mr. M. B., aged seventy-three years, admitted March 7, 1934, to the Philadelphia General Hospital, service of Dr. Wm. E. Robertson, with a chief complaint of vomiting and obstipation.

History.—Prior to the onset of the present illness the patient had been troubled with obstinate constipation. On March 1, 1934, he began vomiting everything he ate, and this condition persisted for a week. The vomitus consisted of a yellow muddy fluid combined with whatever he had eaten. The vomiting occurred at frequent intervals as well as immediately after the ingestion of food. He was able to retain only small amounts of tea and toast. The patient had no bowel movement for several days in spite of the use of citrate of magnesia and castor oil. He was not completely bedfast, but he steadily was growing weaker.

TABLE I

	CHRONIC GLOMERULONEPHRITIS	NONRENAL AZOTEMIA
History of preceding nephritis	May be present	Absent
Blood pressure	Increased	Usually normal or low
Eyegrounds	Usually show exudate hemorrhage or vascular changes	Negative
Respiration	May be Cheyne-Stokes	Usually shallow and rapid but regular
Urine	Acid, low fixed specific gravity, albumin and casts, erythrocytes usually present. Chlorides not significantly altered	Alkaline in hypochloremic type. Sp. gr. variable, usually high. Albumin and casts may be present but disappear with improvement in clinical condition. Erythrocytes not abnormal. Chlorides diminished or absent
Kidney function tests	Always show marked impairment of renal function	May show impairment at height of disorder—improvement occurs with clinical improvement and decrease of blood urea nitrogen
Blood chlorides	Not altered significantly (normal or slightly reduced)	Diminished in hypochloremic coma. Variable in other cases
CO_2 combining power of blood plasma	May be markedly diminished	Increased in hypochloremic cases. Normal or decreased in other types
Blood count and hemoglobin	Reduced	Normal or elevated—anhydremia. One exception in our toxic case—where there was a severe anemia
pH of blood	Usually low	Usually high
Therapy	Course of disease not influenced by administration of salt and fluids	Salt and fluid administration curative in early stages

The man had been in good health previous to his present illness, with no special complaint except constipation for which various laxatives were used. In the two years before the present illness there was no loss of weight, but within a week on becoming sick, the loss was considerable.

Examination.—Here was an elderly man in a deplorable state—there was pronounced emaciation; the skin was dry; the tongue was dry and moderately coated; the eyes were sunken; the man was mentally confused and somnolent.

The blood pressure was 120 systolic and 70 diastolic. The brachial vessels were tortuous and the pulsations visible. Some thickening of the radial vessels was noted.

The abdomen was greatly distended and borborygmus was audible. There was no reverse peristalsis. The size of the liver was within normal limits. The spleen was not palpable. The prostate was large, smooth and slightly tender. No rectal masses were palpable.

The temperature was 98° F. The pulse rate was 80 and the respirations were 25. The tendon reflexes were normal.

The laboratory findings were: blood sugar 132 mg. per cent, blood urea 120 mg. per cent, creatinine 6.2 mg. per cent. The urine was acid in reaction, had a specific gravity of 1.020 and showed a trace of albumin but no sugar. On microscopic examination two-plus leucocytes were reported. The amount of urine voided was diminished.

Treatment.—It was suspected that either an intestinal obstruction or an acute uremia was the cause of the trouble, and therefore an attempt was made to give the man a barium enema but without success. He was then given 3,500 c.c. of 3 per cent glucose in normal salt solution subcutaneously and within twenty-four hours the vomiting stopped. This procedure was repeated the following day. The patient became able to retain fluids by mouth and his mentality began to clear.

The blood chemistry seven days after his admission showed a blood urea nitrogen of 100 mg. per cent, a blood sugar of 104 mg. per cent, and a creatinine of 2.4 mg. per cent. Large amounts of glucose and saline were given by rectum in addition to subcutaneous saline administration. The diuresis increased considerably. On the fourteenth day after admission the blood chemistry showed: urea nitrogen 50 mg. per cent, and creatinine 2.0 mg. per cent. By March 23, improvement continued and the patient was able to sit up in a chair and he was mentally quite clear. By March 25 he was taking food freely and doing well. The kidney function tests and urinalysis were about normal, and the patient was discharged.

CASE 2.—Vomiting following self-treatment with a duodenal tube. Mrs. K. C., aged forty-four years, was examined by one of us (M. G. W.) at the Temple University Hospital, sixteen months prior to her present illness. A diagnosis of noncalculous cholecystitis and general visceroptosis was made. The patient had several so-called nonsurgical gallbladder drainages. She then, without the physician's permission or knowledge, procured a Rehfuss tube which she used whenever nauseated or had, as she put it, a "bilious attack."

On May 5, 1934, the patient vomited a large quantity of dark green material. This vomiting continued for the following five days and she was unable to retain anything on her stomach. She continued to use the Rehfuss tube for the next four days. On the ninth day she suffered a violent headache; she became extremely weak and somnolent. When the woman was seen she looked emaciated; her skin was dry, her eyes were sunken, but the tongue was moist, though coated. The breathing was rapid but shallow and faint breath sounds were heard over the lungs. The heart was rapid and the sounds weak. The blood pressure was 90 systolic and 70 diastolic. The abdomen was greatly distended. The tendon reflexes were retained.

The urine showed a trace of albumin, many cylindroids, and a number of leucocytes. There were practically no chlorides in the urine. The blood chemistry showed: urea nitrogen 75 mg. per cent and chlorides 420 mg. per cent.

Treatment.—Bismuth carbonate gr. v and cocaine hydrochloride gr. ¼ were given every two hours until the gastric irritability was allayed. Rectal administration of saline and 3 per cent glucose was adopted, and in addition the patient was given highly salted bouillon and was instructed to chew salty fish. After twelve hours of this salt diet, fluids and some food could be retained. The woman gradually became stronger and she continued to improve.

Two weeks later the urine showed the presence of chlorides; the blood urea had declined to 22 mg. per cent and the blood pressure had risen to 115 systolic and 80 diastolic, and the patient was able to be up and about.

CASE 3.—Diarrhea. A negro laboring man, aged forty-eight years, was admitted to the service of Dr. David Riesman at the Philadelphia General Hospital on Sept. 9, 1934, with a chief complaint of great loss of weight, severe weakness and diarrhea. He was having six to ten bowel movements a day. His trouble began with a chill while at work as a stevedore.

Examination showed a greatly dehydrated, emaciated, dull and drowsy negro in a critical condition. There was no marked dyspnea, no cyanosis or edema. The temperature was 95° to 97° F. The blood pressure was 97 systolic and 82 diastolic. The blood count showed the erythrocytes to be 4,700,000, the hemoglobin 70 per cent, and the leucocytes 7,300. The urine was acid in reaction. There was no albumin and no sugar; but some hyaline casts and leucocytes were reported. The blood chemistry showed the urea nitrogen to be 72 mg. per cent and the creatinine 2.6 mg. per cent.

Treatment.—Hypodermoclysis of normal salt to which was added 50 c.c. of a 50 per cent glucose solution per 1,000 c.c. was given frequently. Bismuth and paregoric and atropine were administered by mouth.

The man began to react at once and steadily improved. No specific lesion or organisms were discoverable in the bowel. On September 28, ten days after admission, his blood urea nitrogen was only 26 mg. per cent and before his discharge it had fallen to 18 mg. per cent. Unfortunately the chlorides were not determined either in the blood or in the urine.

CASE 4.—Vomiting and diarrhea from intestinal obstruction. A. M., a colored man aged forty-four years, a laborer, admitted to the Philadelphia General Hospital on Nov. 29, 1934, with the complaints of vomiting bloody fluid and pains in the abdomen which began the day before admission three hours after last drinking considerable cheap whisky. At 7 P.M. on November 28 he began to have cramps, and he vomited a "gallon" of brownish black stuff. By midnight he had twelve stools in quick succession without blood. The pain continued.

His past history revealed that he had been operated upon for appendicitis and that "at the same time he had an ulcer cut out through a higher incision."

Examination showed a middle-aged negro man who was restless, slightly cyanotic in his lips, and malnourished. He was oriented, intelligent and cooperative. The skin was warm and moist. The face had an anxious and pinched expression. The abdomen had increased resistance to palpation and tenderness in the upper half. There was no distention and no fluid. The blood pressure was 110 systolic and 80 diastolic. The urinalysis showed an acid reaction, a specific gravity of 1.020. There were two-plus albumin and two-plus leucocytes but no sugar. Blood erythrocytes were 3,530,000; leucocytes 9,400.

By November 30 the patient was very dehydrated. Intestinal obstruction was diagnosed and conservative treatment by the Wangensteen suction siphonage method was instituted. On December 1 the erythrocytes numbered only 2,440,000 with the hemoglobin reading 50 per cent. The blood chemistry was: sugar 109 mg. per cent, urea nitrogen 115 mg. per cent, creatinine 4.5 mg. per cent, and chlorides 374 mg. per cent.

On December 1 he was given salt solution hypodermically and glucose intravenously. He became almost pulseless. The same day he was given 500 c.c. of citrated blood. The next day he improved; the duodenal tube was withdrawn on December 3, but he started to vomit again in spite of continued parenteral salt and sugar solutions.

On December 4 he was operated upon and twelve inches of a jejunum which had been anastamosed to the stomach were found intussuscepted into the latter which was enormously distended. Parenteral salt and sugar solutions were continued. By December 5 his blood urea nitrogen was 60 mg. per cent and the creatinine 2 mg. per cent. The chlorides were now 590 mg. per cent. On December 15, with continued clinical improvement, the urea nitrogen was 12 mg. per cent and the chlorides 545 mg. per cent.

Postoperative hyperazotemia and hypochloremia have already been commented on in the literature[21, 22] and hypertonic (4 per cent) solutions of salt have been recommended intravenously to combat accidents due to these states. That such conditions do sometimes prevail after operation, and the importance of properly diagnosing the pathologic physiology, are the reasons for including the following case.

CASE 5.—Postoperative dehydration. Mr. A. B., a white man aged seventy-three years, a known mild diabetic (not requiring insulin) of at least two years' duration, was admitted

to the Jefferson Hospital on Sept. 24, 1934, on account of acute complete urinary retention from benign hypertrophy of the prostate. His general condition was good for a man of his age.

The laboratory findings on admission were: urine, alkaline in reaction; specific gravity, 1.010; albumin, negative; sugar and acetone, negative. Red blood cells were found as well as an occasional leucocyte. There were no casts. Blood: the count showed erythrocytes, 4,750,000; leucocytes, 9,200; color index, 1.02; hemoglobin, 96 per cent. The Kahn test was negative. Chemistry: urea nitrogen 21 mg. per cent, and sugar 193 mg. per cent. The urine examination subsequently showed a variation in specific gravity from 1.010 to 1.020. On only one occasion, Sept. 16, 1934, was there any albumin, and then a cloud was reported present.

A two-stage prostatectomy was done, the second stage under spinal anesthesia on Oct. 6, 1934. After the first stage the blood sugar was kept between 116 and 129 mg. per cent. On Oct. 3, 1934, the blood nonprotein nitrogen was 29.85 mg. per cent, and the urea nitrogen was 19.85 mg. per cent.

Following both stages glucose 5 per cent in 1,000 c.c. of normal salt solution was given intravenously with one unit of insulin for every gram of glucose. During the second stage there was a severe reaction from the spinal anesthesia but this responded to epinephrine hydrochloride, 1 c.c. of the 1:1,000 solution and ephedrine sulphate ¾ gr. hypodermically.

In the two days following, the patient did not take fluids as he should have and on October 8 his blood nonprotein nitrogen rose to 50 mg. per cent with the creatinine 1.72 mg. per cent, and the patient became drowsy and his skin was dry. Fluids were pushed and by October 11 the nonprotein nitrogen had come down to 33 mg. per cent.

This and another almost identical case (nondiabetic) were at first regarded as impending renal failure following prostatectomy until the true background of the sudden elevation of nonprotein nitrogen was evaluated and counteracted by forcing fluids. Without the immediate postoperative intravenous infusion, no doubt the condition would in both of these cases have been far more alarming, especially on account of the nature of the condition.

Congestive heart failure with passive congestion of the kidneys has been mentioned in the literature as a possible, though by no means likely, cause of great elevation of blood urea nitrogen. Tileston and Comfort[10] reported a study of a series in which one case showed an elevation of 71 mg. per cent. Cases, however, have been known[23] where, due to congestive failure, the clinical picture so resembled uremia of renal origin that the patient was about to be given a hot pack until the cardiac origin of the renal symptoms was stressed. The renal picture reverted to normal on reestablishment of circulatory competence. A milder case came under the observation of one of us (R. B.) recently.

CASE 6.—M. S., a negro man aged forty-four years, with congestive heart failure from hypertensive and/or syphilitic heart disease. He was first admitted to the Philadelphia General Hospital on March 18, 1934, with moderate congestive heart failure and a blood pressure of 160 systolic and 120 diastolic.

The urine showed an acid reaction, a specific gravity of 1.016 to 1.030, a trace of albumin and, on microscopic examination, there were occasional erythrocytes, granular casts and leucocytes in the first specimen only.

The electrocardiogram showed definite evidence of myocardial disease.

The man improved steadily and was discharged only to be readmitted on Nov. 2, 1934, in a worse state than the first time. He had anasarca, a large liver, bilateral hydrothorax and Cheyne-Stokes breathing.

The urine was acid, the specific gravity was 1.015 to 1.030 with, at first, a heavy trace of albumin and some casts. When his condition improved the albumin and casts were no longer present.

On this admission the blood urea nitrogen was 42.0 mg. per cent and the creatinine 2.0 mg. per cent and this urea nitrogen came down to 29.0 mg. per cent after his congestive failure was relieved and he improved generally.

CASE 7.—More severe acute (toxic?) kidney damage apparently not associated with any previous renal lesion or even any glomerular damage is illustrated by the following unusual case:

A colored man, J. D., aged twenty-four years, was admitted to the Philadelphia General Hospital on June 24, 1934, for headache, vomiting, and inability to move his bowels well. There was a gradual onset preceded by a chest "cold" and the drinking of a half pint of "corn whisky." He had nocturia, frequency and dysuria since the onset. He had an acute gonorrheal urethritis one month before.

Examination showed a well-nourished and well-developed young negro man with warm sweating skin and a fever of 101° to 103° F. His blood pressure was 110 systolic and 78 diastolic. He was passing cloudy urine. The man was at first thought to have an intestinal obstruction and was sent to the surgical ward where the report of a blood urea nitrogen of 105.0 mg. per cent suggested uremia, and he was transferred to the medical ward. There were no oliguria, hematuria, or pyuria but there were urinary frequency, urgency, and slight burning on micturition. There were no casts in the urine until July 3, 1934, and the specific gravity ranged from 1.010 to 1.016. At first there was a heavy cloud of albumin but this disappeared by July 4, 1934. On July 3 it was noted that "in spite of daily intravenous glucose his blood urea nitrogen had increased rapidly and progressively" (to 230 mg. per cent). There was urea frost on his face; he was toxic and apathetic.

On July 8 a twenty-four-hour specimen of urine showed a trace of arsenic. By July 10 he had a blood pressure of 140 systolic and 90 diastolic. The phenolsulphonephthalein test showed no elimination of the dye and the Mosenthal test showed a low fixed specific gravity and a night to day ratio of 4/1. The eyegrounds showed nothing abnormal. The blood count showed a severe anemia, the erythrocytes being 1,680,000 and the leucocytes 7,200 with a normal differential count.

On July 22 the man was given an indirect blood transfusion of 200 c.c. By July 28 he felt better and his blood urea nitrogen was only 32 mg. per cent. By August 12 the blood pressure was 130 systolic and 80 diastolic. The phenolsulphonephthalein test showed an output of the dye of 14 per cent in the first hour and 16 per cent in the second hour. He continued to improve and was discharged in good condition with a blood urea nitrogen of only 22.0 mg. per cent. The diagnosis was acute toxic nephrosis.

Table II shows that the blood chlorides in this case were not significantly reduced but that at the height of his blood urea nitrogen he had a marked acidosis.

TABLE II

BLOOD CHEMISTRY

DATE	SUGAR	UREA N	CREATININE	BLOOD CHLORIDES IN TERMS OF NACL	CO_2	SERUM PROTEIN
6/26/34	109	105				
6/28/34	107	150	11.2	586		
7/ 3/34	125	230				
7/ 6/34	114	240	16.0		19 vol. %	Total 6.50 {albumin 3.18 globulin 3.32
7/10/34						
7/21/34	82	32				
7/28/34	96	23				
8/13/34	84	22				

This case was doubtless one of severe renal function impairment but on the other hand, not the type commonly associated with azotemia and more amenable to therapy.

CASE 8.—Epilepsy. Miss L. R., aged twenty-three years, admitted to the Temple University Hospital, Nov. 11, 1933, service of Dr. Temple Fay. The chief complaint was convulsive seizures. The history stated that the girl had suffered her first seizure at the age

of sixteen and since that time there had been one or two a month. These seizures seemed definitely related to the menstrual cycle occurring especially at the onset of the menstruation. They lasted from a few minutes to an hour and were followed by headache.

The case had been diagnosed as one of essential epilepsy, and the patient had been on a fluid limitation and salt-restricted diet for a period of two years. The night before admission to the hospital there were several convulsive seizures, and the following day eight of them. Amytal was given and oxygen inhalations were administered. Two days after admission the patient was stuporous, responding to pinprick only; she did not reply to questions, the pupils were contracted and responded only slightly to light. Physical examination was otherwise irrelevant. The eyegrounds showed no abnormalities.

On November 16 the blood chemistry showed a remarkable finding: the nonprotein nitrogen was 180 mg. per cent and the urea nitrogen was 95 mg. per cent, the creatinine was 7.3 mg. per cent, and the blood sugar was 135 mg. per cent.

The patient was given 3,000 c.c. of normal salt solution intravenously; she reacted well to the saline and was able to answer questions intelligently and to recognize her relatives.

On November 17 the patient's blood count showed a hemoglobin of 14.5 gm. per 100 c.c. The red blood cells were 4,450,000 and the white blood cells were 17,900. The differential showed young forms 38 per cent, neutrophiles 35 per cent, lymphocytes 16 per cent, and monocytes 11 per cent. Urinalysis showed a moderate trace of albumin, but no sugar. Microscopically there were found an occasional hyaline cast and a few white blood cells. The sedimentation time of the red blood cells was within normal range. The spinal fluid had a pressure of 8 mm. Hg and the chlorides were 775 mg. per cent.

The patient continued to be in a semistuporous state with a blood pressure of 95 systolic and 55 diastolic. The pulse rate was 120 per minute and the respiratory rate was 30 per minute. The temperature remained elevated, the highest point reaching 104° F. in the afternoons of the thirteenth and fourteenth of November.

On November 18 the blood urea was 100 mg. per cent, creatinine was 1.7 mg. per cent, and the chlorides were 520 mg. per cent. On that date she again lapsed into coma and was given 500 c.c. of 10 per cent glucose in saline solution intravenously. In addition 1,000 c.c. of saline were given by hypodermoclysis. This treatment was repeated on the following day, and the blood chemistry was then as follows: urea nitrogen 83 mg. per cent and creatinine 1.6 mg. per cent.

On November 21 the patient reacted from coma, answered questions intelligently and was quite cooperative. She complained of being weak. On this date the blood chemistry was: nonprotein nitrogen 56 mg. per cent and urea nitrogen 23 mg. per cent. The temperature had dropped to 100°, the pulse was 70 per minute, and the respirations were 20 per minute.

On November 23 the patient became quite alert and active. The temperature returned to normal and her general condition was good. The blood urea nitrogen on this date was 17 mg. per cent.

On December 1 the blood urea nitrogen was 9.0 mg. per cent and the patient became entirely free from symptoms (Table III).

TABLE III

SHOWING CHEMICAL CONSTITUENTS OF THE BLOOD IN CASE 8

DATE	NONPROTEIN NITROGEN	CHLORIDES	UREA NITROGEN
Nov. 17, 1933	180		95
Nov. 18, 1933		520	100
Nov. 20, 1933			83
Nov. 21, 1933	56		23
Nov. 23, 1933			17
Dec. 1, 1933			9

The electrocardiogram taken during the height of the coma and subsequent to it is worthy of note. During the coma the electrocardiogram showed a slight depression of the S-T interval in Lead II, and this changed on the following day to an inverted T-wave with a depression of the S-T interval. With recovery of the patient the T-wave tended to become flat and later upright.

On December 4 the patient had two convulsive seizures each lasting two minutes and associated with involuntary voiding. She again suffered seizures on the tenth and fifteenth of December. She was discharged from the hospital on Dec. 19, 1933.

DISCUSSION

An analysis of the conditions with which high blood urea nitrogen is associated will reveal the similarity of certain types.

1. Loss of body fluids and depletion of blood electrolytes: (a) by vomiting, by bowel, or by skin. (b) Deficient intake: starvation, deprivation of salt and water.

2. Disturbances in the kidneys themselves: (a) acute intoxication and (b) passive congestion.

3. Neurogenic? (a) reflex anuria and (b) central cerebral?

4. Miscellaneous and little understood disturbances of metabolism.

A review of the cases reported will indicate that by far the commonest clinical cases fall into the first group, all but two of our cases.

Soon after Folin's method of blood analysis came into general use, Tileston and Comfort in 1914[10] reported a study of a miscellaneous series of cases in whom they had made estimations of the blood urea nitrogen and found elevations. They had three cases of acute intestinal obstruction. Cooke, Rodenpaugh and Whipple, in 1916 reported[24] experimental confirmation of high blood urea nitrogen values and more particularly high values for noncoagulable nitrogen in dogs with ligated loops of bowel. In 1919 Porges[25] reported clinical cases of associated high blood urea nitrogen and low blood chlorides. Later (1923) Brown and his associates[26] presented cases of high intestinal obstruction with the clinical picture of shock and coma. These observations were duplicated and further elaborated by Haden and Orr[11] in 1923 and by Whipple, Cooke and Staers in 1927.[27] High CO_2 combining power of the blood was observed by these workers and their animals quickly improved when sodium chloride was administered.

Previously, in 1908, Grunwald[28] had reported that dogs on diuretin and a salt-free diet developed a marked depletion of body chlorides and that as a result the animals went into coma from which they died unless salt was given them.

The symptoms the patient shows depend partly upon the conditions underlying the loss of chlorides and partly upon the state of dehydration. Loss of weight, asthenia, decline of blood pressure, rise in temperature, dryness of the skin, and shallow respirations portray the clinical picture of hypochloremic coma. The patient may also become disoriented, somnolent, and should the chlorides greatly diminish, perhaps comatose. The blood is anhydremic with a high hemoglobin and an increase in red blood cells per unit volume. The nonprotein and urea nitrogen are high. The blood chlorides are perhaps diminished but may be normal, especially in the early stages. The carbon dioxide-combining power of the blood plasma is increased, the urine may show albumin and casts, but a diminution or total absence of chlorides is the most striking finding. Neither the level of the blood urea nitrogen nor of the blood chlorides always determines the onset of coma; this will probably depend upon the constitutional predisposition of the patient, especially with reference to the nervous system.

The important thing is to recognize the condition because the administration of salt solution is so valuable in restoring the patient quickly to a normal state. For its recognition no elaborate procedures are necessary. After all, as Peters[29] states, the clinical estimate of dehydration, dry inelastic skin and mucous membranes, loss of panniculus, inelastic eyeballs, scanty high-colored urine of high specific gravity, etc., are more accurate than laboratory procedures, and the history is usually clear. Furthermore, for the general practitioner the examination of the urine for chlorides and of the blood for hemoglobin when added to the clinical picture will usually suffice to estimate fairly accurately such a case.

Concerning Types II, III, and IV, less definite information is at hand and general principles will have to guide the diagnosis and conduct of such a case.

The association, infrequent as it may be, of severe functional kidney impairment in passive congestion should be borne in mind as well as the recognition of milder phases in most instances of circulatory failure with some evidence of renal damage. These usually clear up more or less completely with restoration of cardiac competency.

Just why the urea nitrogen rises is not clear any more than is the wonderful normal blood regulatory mechanism. "The nonprotein nitrogen content of the blood may be considered as the resultant of three factors: the rate of destruction of protein in the body, the concentrating powers of the kidneys and the amount of water excreted. If the destruction of nitrogen is great enough, as it may be in such profound intoxications as lobar pneumonia, the blood nonprotein nitrogen may rise even when the kidneys are sound, especially if water excretion is small because the patient has received too little fluid."[29]

The story would not be quite complete without mentioning the many points of similarity between hypochloremic coma and the crises of Addison's disease. In both the prominent signs are: weakness, prostration, fall in blood pressure, high blood urea values and low blood chlorides. Sodium chloride alleviates to a striking degree these symptoms and findings in both Addison's disease and hypochloremic states. Out of the many functions, proved and unproved, assigned to the adrenal cortex one fact stands out, namely, that the hormone of the adrenal cortex exerts an influence on sodium metabolism and thus has a regulatory effect on salt and water metabolism.

CONCLUSIONS

1. Attention has been called to clinical states of high blood urea nitrogen values not due to chronic glomerular nephritis.

2. The importance of early recognition of this condition and the prompt improvement with proper therapeutic measures in certain types are illustrated by a number of cases.

3. The mechanism of nitrogen retention in these cases is not well understood, but it would appear to be intimately related in a majority of instances to alterations of salt-water metabolism.

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THE CARDIOVASCULAR SYSTEM IN PROTECTED AND UNPROTECTED ANIMALS WITH ACUTE DIFFUSE PERITONITIS*

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IN A PREVIOUS publication,[1] one of us produced experimental evidence that death in acute diffuse peritonitis is due to soluble toxic substances elaborated by bacteria present in the peritoneal cavity which are responsible for the peritonitis. This contention was based on experiments in which the peritoneal inflammation was induced by intraperitoneal injection of diphtheria bacilli suspended in gum tragacanth. Those animals which were given subcutaneous or intravenous diphtheria antitoxin invariably survived in spite of a marked peritonitis. Animals without antitoxin invariably succumbed.

Holzbach[2] in his review on circulatory failure in infectious conditions states that functional heart failure appears early in peritonitis, but this opinion is apparently based on clinical observation, since no experimental work on this condition was reviewed.

Two types of peritonitis were induced in dogs in our experiments: Fecal peritonitis by the intraperitoneal introduction of 5 gm. of small and large bowel feces suspended in 25 c.c. of saline; and, colon bacillus peritonitis by intraperitoneal injection of 50 c.c. of a 2 per cent gum tragacanth containing 200 million *B. coli* per c.c. Electrocardiographic and carotid pressure tracings on the kymograph before the onset and during the course of the peritonitis were the two criteria employed to determine the effect upon the heart. Two series of animals were employed: one series of twelve dogs in which no protection of any type was administered to the animals; in the other series of six animals all the dogs were protected by the administration intraperitoneally twenty-four to forty-eight hours before the onset of peritonitis of a suspension of killed colon bacilli in 1 per cent gum tragacanth (for the sake of brevity the material will be referred to as "coli-bactragen"). Previous publications[3] describe in detail the technic and the basis of this method of protection with coli-bactragen. It was desired to determine whether such a protection will evince any change which may otherwise be present in the unprotected animals on the electrocardiographic and kymographic tracings. All the animals were under pentobarbital sodium anesthesia. Along with each animal with peritonitis, a normal control dog was run simultaneously.

THE EFFECT OF PERITONEAL INFECTION ON BLOOD PRESSURE IN PROTECTED AND UNPROTECTED ANIMALS

The effect on the blood pressure in the animals with fecal and *B. coli* peritonitis was alike. Within fifteen to twenty minutes after onset of the in-

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fection, the blood pressure began to drop gradually. In one hour there was a drop of 2 mm. of mercury. The fall was of the systolic pressure, the diastolic remained normal. The pulse pressure became progressively smaller. In six hours, the pressure dropped 5 mm. of Hg and at the same time an arrhythmia appeared. In the colon bacillus peritonitis, the fall was still more rapid although it seldom exceeded 4 mm. of mercury.

In the animals protected with coli-bactragen twenty-four to forty-eight hours prior to the onset of peritonitis, the systolic pressure dropped 1 mm. of mercury within ten to fifteen minutes and at the end of ten hours there was a total drop of 2 mm. of mercury. This drop in the protected animals was invariably a third

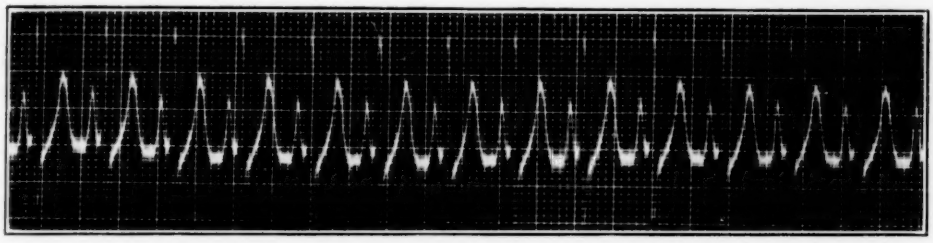

Fig. 1.—Electrocardiographic tracing, one-half hour after induction of peritonitis in an unprotected animal. The pulse rate is 200. (An increase from a normal of 90.) There is some depression of the ST-interval. In this as well as in all the subsequent figures Lead II is shown.

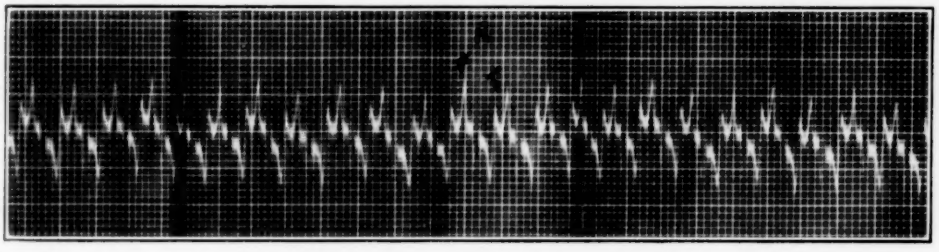

Fig. 2.—Electrocardiographic tracing, six hours after induction of peritonitis in an unprotected dog. The pulse rate is 264. The ST-interval is depressed. The T-wave is diphasic.

or one-half less (in mm. of mercury) than in the unprotected. There was an absence of arrhythmia which was observed in the unprotected dogs.

THE EFFECT OF PERITONEAL INFECTION ON THE HEART IN PROTECTED AND UNPROTECTED ANIMALS AS INTERPRETED FROM ELECTROCARDIOGRAPHIC TRACINGS

Electrocardiographic tracings were taken prior to and at half-hour intervals in unprotected dogs in which peritonitis was induced. The changes in the animals were progressive and rapid. There was a marked increase in pulse rate, from 90 to 220, in the space of four hours. Accompanying the rate increase was a depression of the ST-interval and the appearance of a diphasic T-wave. Six hours after the onset of peritonitis, the pulse rate had increased to 264, and a still greater T-wave deformity appeared. These changes were interpreted to

indicate myocardial damage. Irrespective of the type of peritonitis, whether fecal or colon bacillus, the changes were similar in character.

Six dogs were protected by the administration of coli-bactragen and forty-eight hours later peritonitis was induced. Three of the animals were injected with fecal material and the other three were given colon bacillus peritonitis. Shortly before the onset of the peritoneal infection electrocardiographic tracings

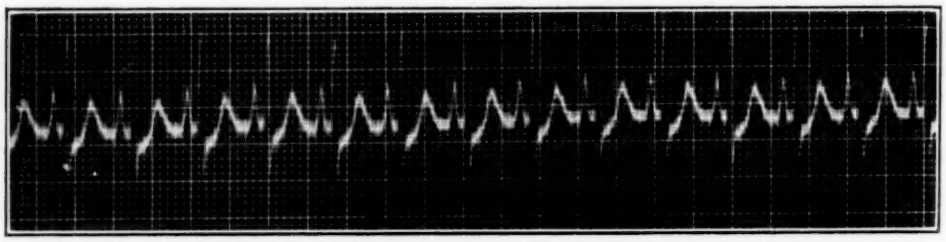

Fig. 3.—Electrocardiographic tracing forty-eight hours after introduction of coli-bactragen and before onset of peritonitis. The pulse rate is 180 (an increase from 100 before introduction of coli-bactragen). There is a slight depression of the ST-interval.

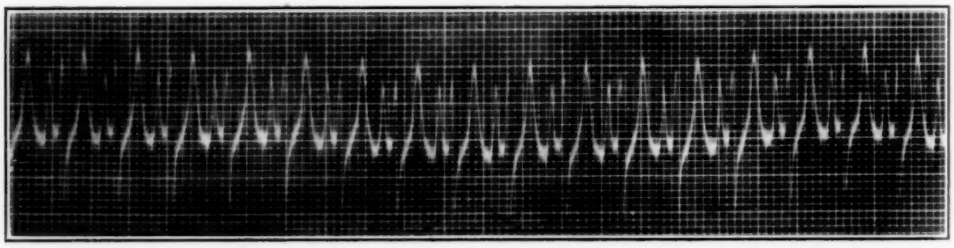

Fig. 4.—Electrocardiographic tracing two and one-half hours after onset of peritonitis in a protected dog. The pulse rate is 210. The complexes are normal.

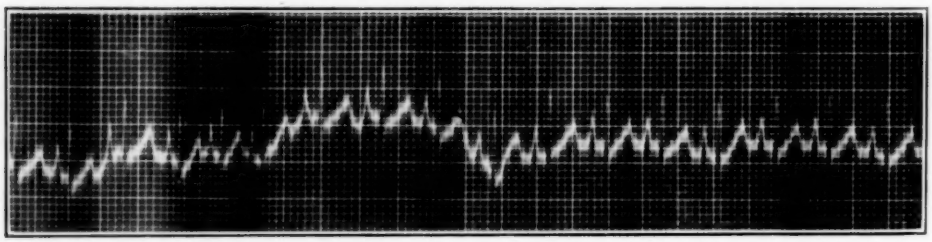

Fig. 5.—Electrocardiographic tracing six hours after onset of peritonitis in a protected dog. The pulse rate is 200. All the complexes are normal.

were taken (forty-eight hours after administration of coli-bactragen). The pulse rate was increased from 100 (before administration of coli-bactragen) to 180. The ST-interval was slightly depressed (Fig. 3). At half-hour intervals following induction of peritonitis, electrocardiographic tracings were made. The pulse rate increased to 210 in two and one-half hours. In six hours the rate was 200. In none of the tracings were there any changes in any of the ventricular or after-complexes which would indicate myocardial damage (Figs. 4 and 5).

DISCUSSION

There are several conceptions regarding the type of organ or tissue upon which bacterial toxic substances elaborated in the peritoneal cavity exert their deleterious action. Manenkow[4] contended that the toxins ascended along the nerve lymphatics and paralyzed the medullary vascular centers. Kirschner[5] suggested that the capillaries of the peritoneum were paralyzed producing stagnation of the portal circulation. Askanazy[6] believed that the toxins dilated the intestinal lymphatics which pressed on the ganglion cells with a consequent paralysis of the bowels. No sufficient convincing experimental evidence has been brought forward to justify any of the above conceptions, although all the suggested tissues and organs may very well be acted upon at the same time by the toxic substances. Death may not necessarily be due to the action upon a single organ or tissue.

Some experimental evidence is presented in this work that indicates the involvement of the heart in acute diffuse peritonitis. The findings, under the conditions of our experiments, do not differentiate, however, myocardial damage due to direct action of the toxic substances from an ischemia which may result from a paralysis of peritoneal capillaries and diminished blood supply to the heart. The fall in the systolic pressure, the rapidity of the heart action, the changes in the ventricular and after-complexes in the electrocardiographic tracings may be interpreted either as a direct action of the toxin on the myocardium or as an indirect effect.

Animals which were protected against the lethal outcome in peritonitis by the administration of coli-bactragen failed to show any deleterious effects on the heart as determined by our criteria. The essential protective factor operating in animals with coli-bactragen consists of a rapid phagocytosis of the invading organisms. In previous articles[1, 7] it was demonstrated that successful protection of an animal depends on a rapid phagocytosis by a sufficiently large number of polymorphonuclears to cope with all the invading bacteria. This phagocytosis occurs within a few hours after the onset of peritonitis and consequently the bacteria are prevented from elaborating soluble toxic substances. This contention in former publications is further verified by experiments presented in this work so far as the effect upon the heart was not apparent in the protected animals.

CONCLUSIONS

Under the conditions of these experiments, myocardial damage was demonstrated in acute diffuse peritonitis with carotid blood pressure and electrocardiographic tracings as criteria.

Animals protected against a lethal outcome from acute diffuse peritonitis by the administration of coli-bactragen, failed to reveal any myocardial damage.

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LABORATORY METHODS

OBSERVATIONS ON INTENSIFYING THE METACHROMATIC PROPERTIES OF CRESYLECHT VIOLET*

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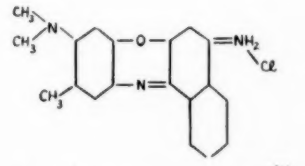
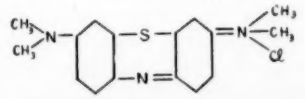
AN INVESTIGATION of the chemistry, physical properties and reactions of the cresyl violets was undertaken in 1922 at the request of the Commission on Standardization of Biological Stains. Several reports have been published.[1-4]

During the early part of 1934, it was decided to change somewhat the methods of study. Up to this time, it had not been regarded as practicable to intensify the metachromasia in a manner similar to that used with methylene blue: this for two very good reasons: (1) it already possesses fairly good metachromatic properties, and (2) it is very susceptible to alkalies even in traces.

Therefore as might be imagined, our first attempts to follow the methylene blue methods were very disappointing. But just as we were about to abandon the venture as one without promise, one experiment showed a very dramatic result. Finally last July a new and improved method for preparing a staining solution was elaborated. During the past six months this has been used routinely in surgical pathology. We have become sufficiently enthusiastic to publish the results, although, of course, further improvements will probably be made.

Contrary to expectations, it has been learned that alkalinization is practicable. However considerable pains must be taken when attempting to intensify: certainly much more care is needed than in the coarse methods used with methylene blue. In the first place, it seems imperative to use a good dye obtained from a constant source, for it has been pointed out[1] that the products of different makers vary greatly in properties. Moreover, as contrasted with the stable methylene blue, cresylecht violet is more unbalanced, delicate, and "fickle." This is observed not only when working with it, but is also suggested by its formula:





*From the Central Office of Williams Pathological Service.
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At any rate, it was decided early in the investigation to avoid heatings. Moreover no matter how hopeless a product might be secured, every effort was made to reclaim it. Chiefly upon these policies success was realized.

In all our experiments the item prepared by the National Aniline and Chemical Company was employed. We have been told that certain men still prefer to use imported cresyl violets. However it has been our experience when studying these foreign makes that no two of them agree in properties; and in our work, an item of constant and dependable composition is imperative.

PREPARATION OF THE SOLUTION

The several steps (as used at present) may be detailed as follows:

First, in a 250 ml. Florence Flask, mix well the following:

Cresylecht violet (National)	1 gm.
Potassium carbonate (dry)	1 gm.
White-water formaldehyde (U.S.P.)	5 ml.
Distilled water	95 ml.

Second, regardless of the physical appearance of the mixture, proceed with the work. Shake at intervals for about one-half hour.

Third, then carefully and in small amounts, add a total of 3 ml. glacial acetic acid. Gas is formed. Contents should be "swirled," or gently shaken in the flask, during the addition of the acid; but the flask should not be corked.

Fourth, swirl occasionally during the next one-half hour and then filter. Add 5 ml. alcohol, propyl, -iso (99 per cent) (Mallinckrodt).

The above first-step yields a brownish-muddy mixture, and it may be felt that, "all is lost." However the addition of the acid brings out a new, intense and beautiful violet. The exact changes we have not been able to determine. For want of a better name, we have been calling the new polychrome "cresyl orchid." However it is probably a composite or mixture rather than a definite substance; and probably we have merely (as with azures in polychrome methylene blue) added traces of minor components to the cresyl violet.

If the alcohol be omitted entirely, the solution will become weaker after a few days, and a reddish substance will be observed clinging to the glass. But prepared with the trace of alcohol, the solution seems to be stable for months. Here where much of it is used, we secure good results by preparing it fresh each month.

Reading over the above description, workers will recognize the rough skeleton of the Goodpasture method for polychrome methylene blue. However no heating operations are employed, the diluents are different, etc.

USE OF THE SOLUTION

The stain is of use in surgical pathology. It is applicable for rapid reading of tissues where prompt diagnoses are urgent. It quickly distinguishes between muscle and fibrous tissues without using elaborate methods like Van Gieson stain, etc. It may be employed with unfixed tissues, but gives excellent results where formaldehyde preparation has been used. It is particularly suited to work which must be rapid and yet accurate (biopsies, etc.) We have

not been able to mount these stains, and their value lies in diagnosis rather than permanent files. The following technic has yielded best results:

First, spread the section on the face of lifter.

Second, drain and gently blot off excess of water, leaving section just moist.

Third, with dropper or pipette, add a drop of the solution or just enough to cover the section.

Fourth, count off about six seconds. Then paddle off the section into a large dish of tap water. (We use a large Pyrex glass biscuit pan for this purpose.) After paddling it around for several seconds (rapid washing), place on a slide in water and add a cover glass.

THE METACHROMASIA

We have been taken to task for our former descriptions of granule staining with cresyl violets. It has been interesting to note, however, that there has been considerable disagreement among the critics. Very likely some of them have been using imported dyes. As a matter of fact, surgical pathologists are not greatly interested in granule staining. Therefore in this report we may wisely ignore the granules and describe the staining reaction of the important elements: *Blue:* all nuclei, muscle cytoplasm; *Pink:* other cytoplasm, fibrils; and *Yellow:* fat cells.

It is rather difficult to imagine a more valuable metachromasia in routine tissue work. Perhaps the stain falls short of expectations in autopsy work and where permanent mounts are wished; but for rapid surgical diagnoses, it is of great promise.

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THE TECHNIC FOR ISOLATION AND PREPARATION OF BACTERIOPHAGE*

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INTRODUCTION.—Because of numerous requests for information concerning the practical laboratory methods of preparing bacteriophage, we will endeavor to present those used in this institution, discussing in turn, equipment and materials employed, sources and manner of isolation, and preparation of stock and specific bacteriophages.

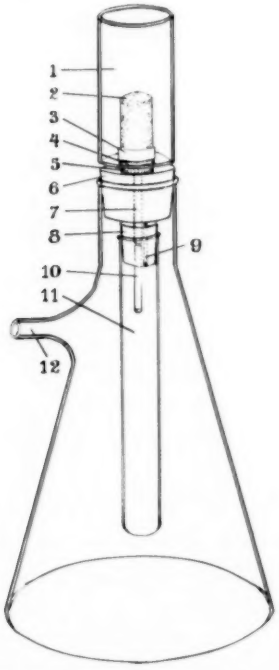

Fig. 1.—Assembled Berkefeld filter. (*1*) Mantle; (*2*) candle; (*3*) metal collar; (*4*) rubber washer; (*5*) metal nut; (*6*) rubber stopper No. 7, with single hole; (*7*) metal stem of filter candle; (*8*) rubber stopper No. 1, with two holes; (*9*) outlet hole through rubber stopper; (*10*) glass tube in continuity with metal stem; (*11*) test tube to receive filtrate; (*12*) side arm of filter flask to connect with suction.

Equipment and Materials.—Among the materials peculiar to this branch of work are filters. After trying various bacteriologic filters, the Berkefeld 3 W and 5 W have been found the most practical. The 5 W filter candles are used for filtering individual set-ups, and the 3 W for the larger volumes. The filters are assembled according to the diagram (Fig. 1). The test tube containing the filtrate may be replaced by an empty sterile tube and the filter used two or

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three times for succeeding generations if the material to be filtered is not too cloudy. Before assembling, new filters are brushed in running tap water and boiled for twenty minutes in distilled water. After using, the parts are boiled separately for twenty minutes in tap water. The mantles are washed and rinsed. The candles are brushed in running water to remove deposits on the outside, and then washed through with suction to remove all traces of medium which have collected throughout the pores, and are finally reboiled for twenty minutes. The parts are allowed to dry and the filters reassembled and autoclaved.

Media.—Two media are commonly used for all the bacteriophage work. The one used for testing and for making bacteriophage to be used locally is meat infusion broth. For subcutaneous and intravenous use, or in case of protein allergy, bacteriophage prepared in a comparatively protein-free medium in which the nutrient is asparagin, one of the amino acid group, is employed.

The asparagin medium[1] is prepared as follows:

Asparagin	3 gm.
Magnesium sulphate	2 gm.
Sodium chloride	4.5 gm.
Bipotassium hydrogen phosphate	2 gm.
Distilled water (neutral in reaction)	1,000 c.c.

Dissolve the substances in water. Bring to a boil, adjust the pH to 7.6. Autoclave at 15 pounds for fifteen minutes. Filter through filter paper, tube, and autoclave again at 15 pounds for thirty minutes. Final pH should be 7.0 to 7.2. A precipitate is usually formed during the first autoclaving. The medium should be clear after the second autoclaving.

Isolation of Bacteriophage.—In isolating a bacteriophage different sources may be utilized. Sewage is the most common source and a bacteriophage for the colon bacillus can almost invariably be obtained. The sewage is filtered through a Berkefeld filter and set up as indicated in Table I.

TABLE I

	ORGANISM CONTROL C.C.	ORGANISM AND SEWAGE FILTRATE		SEWAGE FILTRATE STERILITY TEST C.C.
		4 HOURS C.C.	24 HOURS C.C.	
Meat infusion broth	10.0	10.0	10.0	10.0
Filtered sewage	—	0.5-1.0	0.5-1.0	0.5-1.0
Broth suspension of 24-hour culture	0.1	0.1	0.1	—

The suspension should be heavy enough to make a barely perceptible cloud when 0.1 c.c. is inoculated into 10 c.c. of broth. The tubes are then incubated at 35° C. until there is a perceptible growth in the control, usually about four hours. At that time the preliminary reading is made. An arbitrary scale of clearing has been adopted as follows:

Four-plus lysis	Complete clearing
Three-plus lysis	Slightly cloudy, much less growth than in the control, and with no bacterial sediment
Two-plus lysis	Perceptible clearing, but with some sediment
One-plus lysis	Heavy growth, only slightly clearer than the control.

At this time one tube is filtered, the filtrate placed in the ice box, and the other tubes remain in the incubator overnight. A reading is then made, using the same scale. If the lysis is not four-plus at this time the process is repeated, using the filtrate which has been procured after the four-hour reading. When lysis is complete, a volume of the bacteriophage is prepared, using the same materials and amounts as described above, but setting up a greater number of tubes so that a larger amount can be obtained. It is then titrated by the fluid method described by d'Herelle.[2]

Other sources from which bacteriophage may be isolated include specimens from patients in either chronic, acute or convalescent stages of disease; sludge, animal feces, and impure water. Slight variations in the method of isolating bacteriophage may be employed. The sample from which a bacteriophage is desired may be inoculated with a susceptible strain of organism and incubated twenty-four or forty-eight hours before the first filtration. In isolating bacteriophage from patients' specimens, such as pus, blood or feces, the specimen is placed in nutrient broth and incubated overnight before it is filtered.

The potency of a bacteriophage is ascertained by titration (d'Herelle[2]). A titration of 10^{-8} is required for all stock races, and a higher titer is desirable whenever possible. The titer may be raised in some cases by serial filtration even after four-plus lysis for twenty-four hours has been reached.

Testing of Specimens.—The organisms which are to be tested for bacteriophage susceptibility are isolated and transplanted on agar slants, or where the growth is scant, in broth. Stock bacteriophage is used as the filtrate and the organism is set up and serial filtration carried out by the same method as is used in isolating a bacteriophage (see Table I).

The process is repeated until the lysis is complete, or for five generations, after which, if there is no sign of lysis, or no noticeable improvement, the culture is reported not susceptible or partially susceptible as the case may be. It is important to remember that not all organisms are susceptible to bacteriophage.

If an asparagin bacteriophage is desired, the suspension is made in asparagin, and not more than 0.5 c.c. of broth filtrate is used for 10 c.c. of asparagin medium. Where the bacteriophage is to be used for intravenous injections, it is advisable to carry it for two generations in asparagin, thus reducing the broth concentration to a negligible minimum.

Volumes of bacteriophage specific for the organism are prepared as soon as four-plus lysis, which persists for twenty-four hours, has been obtained. This is done by setting up several tubes with the organisms and the filtrate which gives the four-plus lysis. After four hours this is filtered and sterility tests are made by placing 1 c.c. of the bacteriophage in a tube of nutrient broth and incubating forty-eight hours. If no growth is visible in that time it is assumed that the filtrate is sterile. No bacteriophage which shows the slightest cloud should be used in therapy.

Aseptic precaution must be observed in tubing the bacteriophage, since any air contamination would render it unfit for use. A special room in which all draft is eliminated and which can be washed with an antiseptic is utilized.

The bacteriophage is tubed in amounts applicable for use in the particular case, since after opening the tube it is advisable to use the entire contents. For subcutaneous and intravenous injections the bacteriophage is put up in a vaccine vial with a soft rubber cap.

When a bacteriophage is made up with an organism isolated from a patient's culture it is called "specific" to differentiate it from stock preparations. It is advisable to use this specific bacteriophage whenever possible since it contains, in addition to the lytic principle, the dissolved substance of the invading organism of the patient. Stock bacteriophage is recommended, however, in acute cases, while the specific is being prepared.

Preparation of Stock Bacteriophage.—Stock bacteriophage has a twofold purpose: the testing of organisms and the therapeutic use until a specific preparation is available. It is therefore necessary that it be polyvalent and that it have a high titer. Methods vary in obtaining these qualities for different groups of bacteriophage.

A. *Staphylococcus:* In this laboratory staphylococcus bacteriophage is most widely used. It embodies seven individual races isolated from different sources.* A combination of these phage races in equal parts is set up with a number of virulent and recently isolated staphylococcus strains and filtered together. The new stock is always titrated with one of the strains used in its preparation.

B. *Coli:* Two methods have been found useful in preparing a polyvalent stock coli preparation: first, increasing the number of individual stock races; second, the addition of specific bacteriophages which have been prepared for case strains. The stock coli mixture used in testing and in therapy is a combination in equal parts of forty individual races.† This mixture has been set up with *Coli C.*, the susceptible strain used for the original isolation, in addition to a number of susceptible case strains. Before the mixture is filtered, 10 or 15 c.c. of the specific bacteriophages for each recent case strain are added. Every six months, or more often if the original mixture has become too specific, a new one is made from the original races.

C. *Streptococcus:* Streptococcus bacteriophage is highly specific and four-plus lysis is obtained in but a few of the strains tested. The stock which is used for testing is a combination of six individual races,‡ prepared with a hemolytic and a fecal strain. Since it is not possible to prepare fully potent specific bacteriophage for a majority of the case strains, a partially potent one is often used and has been found of practical use in therapy.

The set-up in Table II is made in duplicate, and one set-up, with exception of the organism control and bacteriophage sterility, is filtered as soon as there is sufficient growth for reading. The duplicate is left in the incubator for a twenty-four-hour reading. The filtrate is then set up in the same manner and

*Staphylococcus races of bacteriophage have been obtained from Bronislawa Fejgin and A. Gratia.

†Coli races of bacteriophage have been obtained from B. R. Callow, Julius Klosterman, Ralph S. Muckenfuss and Gregory Schwartzmann.

‡Streptococcus races of bacteriophage have been obtained from F. d'Herelle, Alice Evans (race isolated by P. F. and A. S. Clark), Norman R. Goldsmith, Helen Jern and Gregory Schwartzmann.

the tubes in which complete lysis occurs are filtered and used in therapy. The remaining tubes are filtered and again set up in the same dilutions to see if a fully potent bacteriophage can be obtained.

D. *Miscellaneous:* The miscellaneous group embodies single races of pyocyaneous, proteus, typhoid, Flexner and Shiga bacteriophages which are prepared with the strains originally used in isolation. Volumes are prepared by using the routine method.

TABLE II

	ORGANISM CONTROL	BACTERIAL SUSPENSION + BACTERIOPHAGE						BACTERIOPHAGE CONTROL
	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.
Meat infusion broth	10.0	—	2.0	6.0	8.0	9.0	9.5	9.5
Bacterial suspension	0.1	0.1	0.1	0.1	0.1	0.1	0.1	—
Bacteriophage	—	10.0	8.0	4.0	2.0	1.0	0.5	0.5

Mixed Bacteriophage.—In cases where a mixed bacterial infection is involved, the bacteriophages are mixed in proportions as indicated by the specific culture. In preparing stock intestinal mixture 100 colitis cases were studied before deciding the appropriate proportion. At the present time it contains about 48 per cent mixed coli bacteriophage, 24 per cent streptococcus bacteriophage prepared with a fecal strain, 24 per cent prepared with a hemolytic strain, and 4 per cent staphylococcus bacteriophage.

No preservative is used in bacteriophage, since the amount necessary to inhibit the growth of bacteria also inhibits the bacteriophage. Aseptic precaution and careful sterility tests are employed. The bacteriophage is kept in the refrigerator and should maintain its potency from four months to three years in this condition. Bacteriophage is sensitive to high temperatures and in incubating the temperature should not be over 35° C. in order to get the best results. Some bacteriophages fail to cause complete lysis if the incubator temperature is increased.

We deem it a privilege to acknowledge the helpful guidance of Professor Ward J. MacNeal and Doctor Adele E. Sheplar, Chief of the Laboratory of Bacteriology, and also the collaboration of our associates in this laboratory.

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PREVENTING AIR HOLES IN SERUM CULTURE MEDIA*

SISTER M. EDELTRUDE PETE, ST. PAUL, MINN.

THE difficulty of producing Loeffler's blood serum culture media without air holes has been a problem to me for sometime, and I discovered it to be such to many others. As a consequence I experimented, and, having found an easy method of offsetting the air hole disturbance, I am offering it to those whom it may interest.

We who have worked in laboratories are well aware that Loeffler's blood serum culture media, as routine media for common culture, have been used with great success, but considerable expense when purchased ready for use. We know also, that to produce these media without bubbles and for long preservation of moisture, is a problem. A little knowledge of the cold-pack and steam-pressure method of canning brought this idea to me; why not use that procedure for culture media?

I mixed the culture media according to the direction given for the Loeffler's blood serum, and put it into small vials or bottles (17 by 60 mm.), using for plugs, corks to about one-sixteenth of an inch from the top of the bottle, and screwing over them tightly fitting aluminum cork-lined caps; and on some others I screwed the aluminum cork-lined caps without the corks. I placed both kinds of vials into the autoclave in a slanting position following the directions for the coagulation and sterilization. Upon removal from the autoclave I found the vials entirely free from air holes or bubbles, the media sufficiently thick and with a smooth surface, plus sufficient water condensation in the bottles to assure indefinite moisture while the bottles were kept in a cool place.

While the cork of a vial remains tight in the autoclave, the expansion of the media during the heating process will cause the rising air to remain in a layer just below the cork, thus preventing bubbles, while the pressure from without keeps the bottles from breaking. If, however, this rising air in the bottle, under the pressure of heat, is allowed to escape from the bottle, the change of pressure within the vial will cause air holes in the media, and sometimes breakage of bottles.

The culture media prepared from Loeffler's blood serum with the aluminum cork-lined caps, which I have described, are so much less expensive than what we had in use, that we are using the former freely now for all of our common routine culture media.

These vials, cork-lined aluminum screw caps, 17 by 60 mm., are $0.35 per dozen, or $3.50 per gross, and can be obtained from the Arthur Thomas Company, Philadelphia.

*From St. Joseph's Hospital.
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BAGG ALBINO MICE FOR TESTING HORMONES AND OTHER EXPENSIVE SUBSTANCES*

J. F. McClendon, Ph.D., and Harold Street, M.S., Minneapolis, Minn.

MICE have been used both by American and European workers in assaying theelin and other substances. The use of mice is particularly advantageous when the amount of hormone in the blood is to be assayed, and yet many workers are prejudiced against the use of mice. They claim mice are delicate, and for nutrition studies the growth rate is too slow. On the other hand, I have found mice quite hardy, standing travel for many thousands of miles in winter weather and living in cold rooms. This is especially true of wild mice who live in unheated shacks with an outside temperature of 30° below zero. I have found the mice that were delicate were infected with some epidemic. Hot weather, however, is more detrimental and care must be taken that they have water at all times and, though very little oxygen is needed, a slight amount of ventilation is necessary for evaporation of moisture in heat regulation.

Bagg albino mice have been used by a number of workers, and it seems to be the most homozygous strain obtainable. From the work of Fortuyn on the albino mouse, and Palmer and his students on rats, it would seem that in selecting for homozygosity, it may be necessary to pay special attention to the particular character whose variability it is desired to reduce.

I have bred Bagg albinos for a number of years and established colonies not only in Minneapolis but also in Sendai and Sapporo, Japan. They grew well in rooms at 40° F. when the metal cages were surrounded by thin paper. They grew well on diets used for rats and developed rickets on Steenbock's diet No. 2965. Harold Street and I have determined their variability in body weight on the following diet:

Ground whole wheat	40.0%
Ground whole flax seed	4.5
Whole milk powder	45.0
Iodized salt	2.2
Yeast	3.3
Alfalfa dust	5.0

as given in Table I, in which 30 to 50 mice of each age and sex were weighed. The coefficient of variability is slightly greater than that observed by King in Wistar albina rats (about the same as in Norway rats) but is not sufficiently greater to warrant the use of rats for very rare substances. Even when the growth rate is taken as an index of physiologic test, it is not the amount of growth which determines their worth but their variability. It would be desirable for

*From the Laboratory of Physiological Chemistry, University of Minnesota.
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TABLE I

BAGG ALBINO MICE: COEFFICIENTS OF VARIABILITY; MEANS AND PROBABLE ERRORS OF MEANS IN GRAMS

DAY OF AGE	MALES			FEMALES		
	MEAN WEIGHT	COEF. VAR. %	PROBABLE ERROR OF MEANS	MEAN WEIGHT	COEF. VAR. %	PROBABLE ERROR OF MEANS
1	1.31	15	0.0246	1.30	15	0.0228
2	1.51	16	0.0306	1.52	13	0.0231
3	1.75	16	0.0314	1.77	18	0.0317
4	2.09	19	0.0409	2.16	15	0.0317
5	2.45	19	0.0501	2.57	17	0.0449
6	2.88	19	0.0598	3.02	18	0.0567
7	3.30	17	0.0631	3.47	23	0.0805
8	3.77	18	0.0736	3.97	19	0.0784
9	4.21	18	0.0841	4.43	20	0.0921
10	4.67	17	0.0843	4.92	20	0.1006
11	5.02	19	0.1038	5.32	21	0.1138
12	5.39	21	0.1236	5.63	23	0.1327
13	5.78	20	0.1288	6.04	22	0.1354
14	6.10	19	0.1272	6.48	22	0.1438
15	6.41	19	0.1315	6.64	23	0.1562
16	6.57	16	0.1164	6.92	21	0.1521
17	6.78	17	0.1294	7.04	22	0.1596
18	6.85	22	0.1645	7.17	21	0.1562
19	6.96	21	0.1621	7.24	23	0.1684
20	7.13	21	0.1605	7.41	23	0.1702
21	7.33	20	0.1676	7.72	21	0.1741
22	7.67	21	0.1812	7.95	22	0.1856
29	9.48	19	0.2601	9.87	19	0.2050
36	12.36	19	0.2601	12.50	18	0.2477
43	14.74	15	0.2588	14.47	15	0.2532
50	16.74	15	0.2860	15.61	15	0.2581
57	18.54	16	0.3496	16.64	14	0.2739

TABLE II

ALBINO MICE, MEAN WEIGHT IN GRAMS

AGE	ROBERTSON 1916	ROBERTSON AND CUTLER 1916	ROBERTSON AND DELPRAT 1917	FORTUYN WM	FORTUYN HTM	GATES MALES	GATES FEMALES	MC CLENDON AND STREET MALES	MC CLENDON AND STREET FEMALES
Birth	1.23	1.47	1.47	1.31	1.41	1.38	1.38	1.31	1.3
1 wk.	3.31	2.99	3.35	3.48	3.97			3.77	3.97
2 wk.	5.14	5.09	4.44	6.03	6.24	5.49	5.64	6.41	6.69
3 wk.			5.89	8.65	8.74			7.67	7.95
4 wk.			8.55	11.64	12.07			9.48	9.87

purposes of correlation of the work of different investigators if more workers on mice used the same breed just as has been done in the case of the Wistar rat.

Table II shows the variation one obtains in using different breeds of mice.

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A CONVENIENT APPARATUS FOR RAPID AND ACCURATE TUBING OF CULTURE MEDIA*

ELIZABETH S. PORTER, M.S., AND RALPH MCBURNEY, M.D., UNIVERSITY, ALA.

THE simplicity and convenience of this device are its chief recommendations, especially where large quantities of media are to be quickly tubed. It enables one to fill a basket of tubes to the same approximate level without moving the tubes, and with more convenience than by holding three or four tubes in the hand and transferring them from one basket to another. Uncomfortable handling when tubing hot agar is eliminated.

Any carpenter can make the wooden structure which may then be finished in dark oak or black. Necessary parts may be used from laboratory stock.

A general view of the boxlike structure is shown in Fig. 1. Detailed features of construction are shown in Fig. 2.

The wooden structure is essentially a box. It is 10 inches wide and 9¼ inches deep at the base. At a height of 7 inches the frame is "stepped" back 4½ inches, on which level is fastened a ¾ inch board, 11 inches long, and 4¾ inches wide, forming a shelflike stage upon which a wire basket of tubes is placed during the tubing of media. Each side is 4¾ inches deep above this level, rising to a height of 9 inches, making the back 16 inches high. It is topped with a ¾ inch board similar to that used in the construction of the stage. It is held in place by small hooks and screw eyes on each side, allowing easy removal for replacing the electric light bulb used for illumination and described later.

*From the Department of Bacteriology, School of Medicine, University of Alabama. Received for publication, February 23, 1935.

In the front, level with the top of the stage, is a window 5½ inches wide and 6½ inches high. It is fitted with ground glass held by 7/16 inch moulding. The whole is recessed to be in the same plane with the sides.

Inside the box, behind the window, is a movable shade which is raised or lowered by a set screw (*a*) (Fig. 2). The shade is made of a piece of sheet tin or aluminum 3 by 6½ inches and suspended by two pieces of wrapping twine tied to a hole punched in each end of the sheet metal, the free ends being knotted through ⅛ inch holes drilled in a ⅜ inch brass crossbar. The holes in the bar are spaced so the strings will hang parallel. The brass bar is made of hollow tubing and is held in place by pushing through holes drilled in each side,

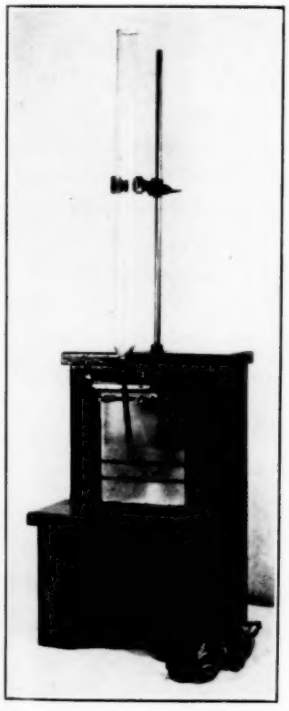


Fig. 1.

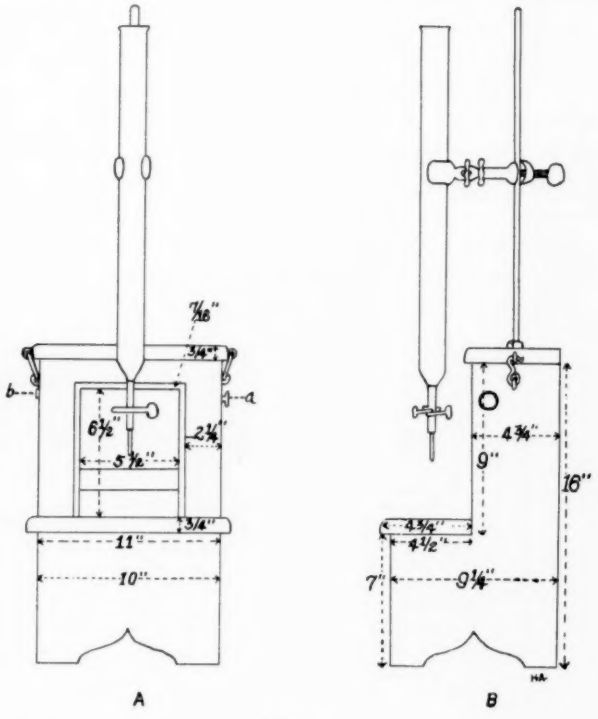


Fig. 2.

about ¾ inch from the front and just above the level of the ground glass window. The holes should be a little larger than the crossbar to allow easy turning. The set screw (*a*) (Fig. 2), used for raising and lowering the curtain, is threaded onto the outside of the bar. At *b* the bar is held by a small metal washer with a machine screw threaded into the tubing.

To prevent the metal curtain from swaying backward a small piece of copper or iron wire is stretched from side to side behind it, about two inches below and in the same plane with the metal crossbar.

A porcelain electric light socket is fastened vertically to a small wooden strip on the inside of the box extending from side to side and approximately level with the height of the stage. Ordinary electric lamp cord is connected to this through the bottom and coming out at either side underneath. A hand

switch may be placed between this and a connection for plugging into any light socket. A 40-watt frosted bulb is ample for illumination. The interior of the box is painted white.

Fastened vertically into the center of the top is an 18 by ⅜ inch iron rod. It is fastened by a nut on both sides of the board with iron washers between and loose enough to allow for free swing of the buret attached by an iron clamp as shown in Fig. 2.

The buret into which media are poured, for tubing, may be adjusted to a convenient height by means of the iron clamp. The buret used in this instance has a capacity of 250 ml. It may be removed for washing, and may be wrapped in paper and sterilized, if necessary for any special media.

Rubber tubing with a glass tip is attached to the end of the buret, for distributing media to the tubes. This rubber tubing should be long enough to reach each tube comfortably. It is fitted with a pinch-cock clamp to control the flow of media.

On the window are two horizontal lines to indicate the levels to which tubes are to be filled for broth cultures, agar slants, and pours. The lines may be drawn with an ordinary blue grease pencil used for marking glassware. The operator sits so that his eyes are on a level with one of these lines. Illumination is from the light inside the box. The ground glass of the window and the shade, which is adjusted to eye comfort, prevent glare.

A basket filled with tubes is placed upon the stage. Tubes are filled in rows beginning at the back and working forward. This permits the operator to see the guide lines for filling.

With the free swing of the buret bar and the rubber tubing and height of the buret properly adjusted, no trouble will be experienced in conveniently reaching every tube.

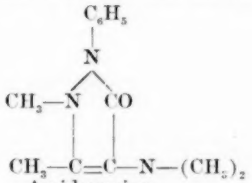
A LABORATORY STUDY OF AMIDOPYRINE, BARBITAL, PHENYL HYDRAZINE, AND BENZENE IN RELATION TO AGRANULOCYTIC ANGINA*

V. L. Bolton, A.B., Kansas City, Kan.

AGRANULOCYTIC angina was described by Schultz[1] in 1922, with the suggestion that an unknown chemical agent or microorganism might be the cause of the disease. Recent opinion points to an explanation involving the effects of chemical substances, represented by a number of drugs.

During the past twelve years administration of the benzene, pyrazolone and barbituric acid derivatives has been associated with the occurrence of the disease in man. Suggestive results also have been reported with reference to the experimental production of granulocytopenia in animals, by the use of amidopyrine, benzene, and the barbiturates.

The clinical evidence emphasizes amidopyrine as a possible etiologic factor in the production of agranulocytic angina. Amidopyrine is dimethyl-amino-phenyl-dimethyl-pyrazolone, a compound containing phenylhydrazine, imino groups, pyrazolone, and the benzene nucleus as potentially toxic fractions.



Amidopyrine

In the following experiments are reported the effects of amidopyrine, barbital, amidopyrine and barbital, phenylhydrazine, and benzene on the cellular elements of the blood of normal dogs. Both the total cell count and differentials were done on venous blood. The results were checked by Miss Esther Rodewald, technician in the hospital laboratory.

RESULTS

Experiment I.—Small doses of amidopyrine.

Dog: Weight 10 kg. Total white count 13,000; with 80 per cent neutrophiles, 16 per cent lymphocytes, 2 per cent monocytes, 1 per cent basophiles, and 1 per cent myelocytes.

June 18: 0.3 gm. of amidopyrine, in water, was administered by stomach tube each day for twenty-one days, a total of 6.3 gm. The white count increased to a range of 18,000 to 20,000; it remained elevated with no significant change in the differential.

July 9: 3 gm. was introduced by stomach tube.

July 10: 0.5 gm., in 5 per cent solution, was injected hypodermically.

July 11: Dog died; 9.8 gm. of amidopyrine had been given, about 1 gm. for each kilogram, in a period of twenty-three days.

*From the Department of Pharmacology, University of Kansas, School of Medicine.
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Necropsy: Sections of the bone marrow of the femur, humerus, and ribs, of the liver, kidney, and spleen were made. There was hyperplasia of the bone marrow, with no decrease in the myeloid elements; some fatty change in the liver; interstitial infiltration of mononuclear cells in the kidneys; congestion of the spleen, with increase of red cells in the sinusoids and prominence of the malpighian bodies.

Experiment II.—Large doses of amidopyrine.

Dog: Weight 10 kg. Total white count 12,200; with 86 per cent neutrophiles, 12 per cent lymphocytes, 1 per cent eosinophiles, 1 per cent myelocytes.

June 18: 1 gm. of amidopyrine, in water, was given by stomach tube each day for seven days, a total of 7 gm.

June 25: 2 gm. were then given each day for nine days, a total of 18 gm.

July 3: 3 gm. were given each day for four days, a total of 12 gm. This represents 37 gm. of amidopyrine, orally; or 3.7 gm. for each kilogram in a period of twenty-five days.

July 7: differential count: 86 per cent neutrophiles, 8 per cent lymphocytes, 2 per cent basophiles, 2 per cent myelocytes, 1 per cent eosinophiles, 1 per cent monocytes. There was a considerable number of normoblasts in the film.

July 8: 8.3 gm. of amidopyrine, 5 per cent aqueous solution, in doses ranging from 0.4 gm. to 1 gm. daily, were given intravenously, in a period of thirteen days. During this interval the total white count began at 12,000; dropped to 6,650 on the third and fifth days of injection; then gradually returned to 12,000 on the thirteenth day of injection. No important change in the differential count was observed.

There had been given 45.3 gm. of amidopyrine, or 4.53 gm. for each kilogram over a period of thirty-three days.

Experiment III.—Sodium barbital.

Dog: Weight 10.5 kg. Total white count 15,000; with 88 per cent neutrophiles, 10 per cent lymphocytes, 2 per cent monocytes.

June 18: 0.5 gm. of sodium barbital, in water, was given by stomach tube each day for fifteen days, a total of 7.5 gm.

July 3: 1 gm. was administered each day for five days, a total of 5 gm. This represents 12.5 gm. of sodium barbital, or about 1.2 gm. for each kilogram, over a period of twenty days.

The minimum total white count was 11,000 on July 7; with 83 per cent neutrophiles, 10 per cent lymphocytes, 4 per cent myelocytes, 2 per cent basophiles.

Experiment IV.—Amidopyrine and sodium barbital.

Dog: Weight 9 kg. Total white count 12,000; with 83 per cent neutrophiles, 13 per cent lymphocytes, 2 per cent monocytes, and 2 per cent myelocytes.

June 18: 0.3 gm. of amidopyrine and 0.5 gm. of sodium barbital, in water, were given by stomach tube each day for fifteen days; a total of 4.5 gm. of amidopyrine, and 7.5 gm. of sodium barbital. The white count increased to 18,150; with no appreciable rise in neutrophiles.

July 3: 1 gm. of amidopyrine and 0.5 gm. of sodium barbital were given each day for seven days, a total of 7 gm. of amidopyrine, and 3.5 gm. of sodium barbital.

July 10: 3 gm. of amidopyrine and 1.5 gm. of sodium barbital were given each day for seven days, a total of 21 gm. of amidopyrine and 10.5 gm. of sodium barbital.

July 18: 0.5 gm. of amidopyrine, 5 per cent solution, was injected hypodermically.

July 19: 0.5 gm. of amidopyrine, 5 per cent solution, was injected hypodermically.

July 20: 1 gm. of amidopyrine, 5 per cent solution, was injected hypodermically.

This represents 28.5 gm. of amidopyrine, 3.2 gm. for each kilogram; 21.5 gm. of sodium barbital, 2.4 gm. for each kilogram, in a period of thirty-two days.

The total white count decreased gradually to 10,000; then returned to 13,000. There was no important change in the differential count.

Experiment V.—Phenylhydrazine.

Dog: Weight 16.2 kg. Total white count 12,200; with 83 per cent neutrophiles, 14 per cent lymphocytes, 2 per cent myelocytes, 1 per cent basophiles. Red cell count 5,550,000; hemoglobin 84 per cent by the Haden-Hausser hemoglobinometer.

June 18: 0.3 gm. of phenylhydrazine hydrochloride, in water, was given by stomach tube each day for four days, a total of 1.2 gm. or 74 mg. for each kilogram in a period of four days.

June 21: Red cell count 4,190,000.

June 25: Red cell count 1,160,000; hemoglobin below 18 per cent.

June 26: A total white count made daily showed a gradual increase to 45,000; with 91 per cent neutrophiles.

July 3: The red cell count gradually increased to 4,930,000 and the total white count decreased to 12,000.

At no time was the granulocyte count below the control level.

Experiment VI.—Benzene.

Dog: Weight 17 kg. Total white count 13,000; with 81 per cent neutrophiles, 15 per cent lymphocytes, 4 per cent myelocytes. Red cell count 5,000,000; hemoglobin 84 per cent.

July 5: Benzene, 50 per cent in olive oil, was given in capsules in doses of 2 to 12 c.c. each day for thirteen days, a total of 80 c.c. of benzene or 4.7 c.c. for each kilogram.

July 13: White count 8,150; with 67 per cent neutrophiles, 24 per cent lymphocytes, 4 per cent myelocytes, 3 per cent eosinophiles, 2 per cent monocytes.

July 14: Red cell count 4,900,000.

July 20: Total white count 13,000.

DISCUSSION

In this series of experiments, definite leucopenia was observed only in those dogs which received large doses of amidopyrine, or of benzene. Absence of significant changes in the granulocyte ratio in the differential count makes it impossible to state that a blood picture comparable to that of granulocytopenia was produced in normal dogs. Attempts to reproduce the disease experimentally by Madison and Squier,[2] Hoffman, Butt and Hickey,[3] and by Kracke[4] have been partially successful.

The present experimental evidence and clinical observation on the relation of drugs to granulocytopenia require further theoretical analysis. This analysis must involve a discussion of the chemistry of amidopyrine and of the barbiturates in relation to their pharmacologic actions.

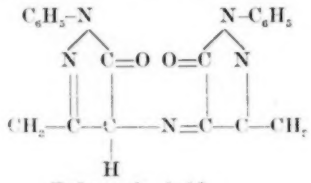
Amidopyrine is dimethyl-amino-phenyl-dimethyl-pyrazolone. Its synthetic precursor, antipyrine, is produced by the condensation of methyl-phenylhydrazine with acetoacetic ester; to antipyrine a dimethyl-amino group is added. There are, therefore, three possible toxic fractions in amidopyrine: pyrazolone, the nitrogen radicals, and methyl-phenylhydrazine. The benzene nucleus found in phenylhydrazine might also be considered injurious, but it should be emphasized that this nucleus is the phenyl group, which is unlike pure benzene.

The action of pyrazolone on the cellular elements of the blood is not described by Fränkel,[5] but its close relation to pyrrole, a toxic substance, may be significant.

Tertiary nitrogen, as found in amidopyrine, is the least toxic of the basic nitrogen radicals. It is potentially toxic in as much as reduction in the body may produce a true imino group. This group has been reported toxic by Herz,[6] but it is not necessarily toxic for myeloid elements.

Fränkel[5] states, according to Joffe, rubazonic acid appears in the urine after the use of amidopyrine.

Its structure indicates a demethylation of all the methylated nitrogen groups. Since McGuigan[7] states that following the use of amidopyrine, rubazonic acid occurs in the urine after standing, and produces a red color due to oxidation,



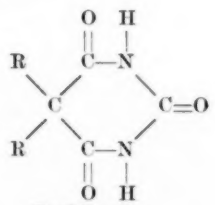
Rubazonic Acid

the formation of rubazonic acid in the body from amidopyrine is questionable. Amidopyrine is also excreted in the urine as a paired glycuronic acid, and as antipyril urea.

In spite of the toxic capacity of the imino group as such, there still remains the more serious possibility of blood destruction by methyl-phenylhydrazine as an hydrolytic cleavage product of amidopyrine.

It is generally accepted that the erythrocyte and granulocyte arise from a common stem cell, the hemocytoblast. In granulocytopenia there is anemia as well as leucopenia. In view of this fact, it would appear that the toxic action of phenylhydrazine, or an unrecognized modification, may in some people be extended to the stem cell or to an early form in the process of its maturation.

The importance of the barbiturates, as stated by Watkins,[8] has not been explained. Herz has suggested that the toxicity of amidopyrine is based on the NH radical, and that the attached benzene ring is insufficient to neutralize its toxicity. Recognizing the superior toxicity of NH over tertiary nitrogen, the barbiturates should be more toxic than amidopyrine, since two NH groups are found in the barbiturate ring, whereas no incompletely substituted nitrogen atoms are present in amidopyrine.



Barbiturate

On the contrary, the prevalence of barbiturate medication as a hypothetical factor in the etiology of the disease is relatively slight in comparison with that of amidopyrine.

Benjamin and Biederman[9] feel that the disease is probably due to a drug hypersensitivity, with the blood system acting as the seat of the shock. Pepper[10] has also called attention to drug allergy in leucopenia. These hypotheses are supported by the fact that many drug hypersensitivities occur, in some of which the formed elements of the blood are affected.

In agranulocytic angina the blood changes are the most important findings. It is logical, therefore, to assume that hypersensitivity may not be the only

factor, since it is known that amidopyrine and the shorter acting barbiturates are altered in the body. These drugs contain fractions which may exert a toxic action in the process of their alteration.

It is apparent, then, that more than one type of reaction and numerous chemical substances could be involved in the etiology of agranulocytic angina. Experiments are in progress in this department to determine the nature of this reaction and of these chemical substances, with special attention directed to the effect on myeloid maturation.

SUMMARY

1. A study has been made of the effects of amidopyrine, barbital, phenylhydrazine, and benzene on the blood count in a series of six dogs.

2. A moderate leucopenia was observed in one animal which received amidopyrine, and in one which received benzene. Anemia with leucocytosis was observed in the animal which received phenylhydrazine. Pathologic findings were not conclusive.

3. It is suggested that in some people transformation products possessing toxic properties are formed; and that by virtue of individual hypersensitivity, these toxic properties result in arrested maturation of the myeloid elements.

The author wishes to express his gratitude for the valuable aid and advice received from Dr. Robert M. Isenberger, Dr. J. C. Rice, and Miss Esther Rodewald.

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SELENIUM AS A CATALYST IN THE DETERMINATION OF NONPROTEIN NITROGEN IN BLOOD*

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SELENIUM was first introduced as a catalyst in Kjeldahl digestions by M. F. Lauro,[1] and its use as such suggested to us the possibility of its being used in the determination of nonprotein nitrogen in blood filtrates, our aim being to develop a method of digestion as rapid and as convenient as the Folin-Wu method[2] but without the troublesome formation of a precipitate of silica, due to the action of the phosphoric acid of their digestion mixture on glass. While this precipitate may be avoided by a careful regulation of the flame, its occurrence is always possible, and when present, interferes with colorimetric readings unless removed by centrifuging.

As a result of several preliminary experiments, it was found that 45 per cent sulphuric acid containing a small amount of selenium could be substituted for the Folin-Wu digestion mixture with very little sacrifice of the length of digestion time, and with no formation of silica precipitates. Either selenium or selenious acid may be used. The best results were obtained with a solution of 70 mg. selenium in 200 c.c. of 45 per cent sulphuric acid.

The digestion is carried out in much the same way as the Folin-Wu method. In place of the charring seen in the latter method, a rust-colored precipitate of reduced selenium appears. This dissolves as the heating is continued, the digest becoming practically colorless within thirty seconds to three minutes after the appearance of the fumes of sulphur dioxide. Five minutes of heating from the first appearance of the acid fumes is sufficient for the complete digestion of most urine samples, and for all blood filtrates unless these filtrates, through improper preparation, contain traces of protein. In such filtrates in the Folin-Wu method, the protein becomes charred and sticks to the side of the digestion tube so that it is not completely digested. In the selenium sulphuric acid method this trace of protein goes into solution, but is quite difficult to digest. Usually an additional five minutes' heating completes the digestion in these cases, and if a slight brown color still persists after ten minutes' digestion, it may be disregarded as it apparently does not affect the accuracy of the determination.

Using the selenium sulphuric acid digestion method for the determination of total urinary nitrogen, analyses were done on urine and on known solutions of urea and uric acid. For the urine a value of 10.7 gm. nitrogen per liter was obtained as compared with 10.64 gm. with the Folin-Wu method. The urea solution gave a value of 3.35 mg. nitrogen per 100 c.c. as compared with the

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calculated value of 3.5 mg. A lithium carbonate solution of uric acid on one analysis gave a value of 3.29 mg. nitrogen per 100 c.c. and on a second analysis, 3.33 mg. as compared with a calculated value of 3.33 mg.

TABLE I

NONPROTEIN NITROGEN OF BLOOD SHOWING THE COMPARATIVE VALUES OBTAINED BY THE FOLIN WU AND SELENIUM SULPHURIC DIGESTION METHODS

SAMPLE	NONPROTEIN N, MG. PER CENT	
	FOLIN-WU	SELENIUM SULPHURIC
1	31.1	31.1
2	35.9	36.4
3	32.4	32.4
4	31.1	31.1
5	34.5	34.7
6	45.5	45.8
7	39.4	39.8
8	37.0	36.9
9	45.6	45.4
10	35.9	35.7
11	38.7	38.7
12	159.1	157.8
13	27.7	27.3
14	22.9	24.3
15	32.2	31.1

Tungstic acid filtrates, especially those from unlaked blood, usually contain varying traces of unprecipitated tungstic acid. In the Folin-Wu digestion the phosphoric acid keeps the tungstic acid in solution, but in procedures not using phosphoric acid, it separates out and results in turbidity on nesslerization. This turbidity may be prevented by the use of gum ghatti solution which was first introduced by Folin[3] in his ferricyanide sugar method and later used by several different authors in methods involving nesslerization. By the use of gum ghatti we have succeeded in nesslerizing solutions that were milky with precipitated tungstic acid, although in some cases the solution would have to stand for several minutes before all of the tungstic acid would be dissolved by the alkali of the Nessler's.

METHOD

The method as outlined is for the determination of nonprotein nitrogen in the regular Folin-Wu filtrates. In the Folin-Svedberg micromethod[4] in which the blood is unlaked, the selenium sulphuric acid solution described below is diluted 1 to 5 and used in place of the regular digestion mixture, and 1 c.c. of gum ghatti solution is added prior to nesslerization. In the determination of the total nitrogen of urine the only change from the Folin method is in the substitution of the selenium sulphuric acid solution.

Solutions Required.—

1. Standard ammonium sulphate solution containing 0.1 mg. nitrogen per c.c.
2. Nessler's reagent.
3. Gum ghatti solution.

Directions for the preparation of the above solutions may be found in *Laboratory Manual of Biological Chemistry*, Folin (1934).

4. Selenium sulphuric acid solution: Dissolve with heat 70 mg. selenium in 90 c.c. of sulphuric acid, cool and dilute to a volume of 200 c.c. with water. It may also be prepared by adding 5 c.c. of 2 per cent selenious acid to 200 c.c. of 45 per cent sulphuric acid.

Determination.—Transfer 5 c.c. of the Folin-Wu filtrate corresponding to 0.5 c.c. of whole blood to a Pyrex test tube graduated at 50 c.c. Add 1 c.c. of the selenium sulphuric acid solution and an antibump tube. Then adjust the flame of a microburner to a height of about one inch and clamp the Pyrex tube so that the bottom is about ¾ inch above the top of the burner. This amount of flame will insure a rapid boiling away of the water and need not be changed during the course of the digestion. When the fumes of sulphur dioxide appear, cover the mouth of the tube with a watch glass and continue the heating for five minutes, or ten minutes, if necessary. Allow to cool thoroughly and add about 25 c.c. of water.

Now place 1.5 c.c. of the standard ammonium sulphate solution containing 0.15 mg. nitrogen in a similar test tube, add 1 c.c. of the selenium sulphuric acid solution and about 25 c.c. of water. Then add 2 c.c. of gum ghatti solution to each, mix slightly by twirling the tubes, add 15 c.c. of Nessler's solution to each, dilute to the 50 c.c. mark and mix. Note: We have found it best to complete the nesslerization and mixing of each tube separately, for if a tube is left even a short time without mixing, the localized concentration of the alkaline Nessler's solution may be sufficient to start the development of turbidity.

The solutions are allowed to stand for about five minutes and then compared in the colorimeter. The calculation is the same as in the regular method: $\frac{20}{\text{reading of the unknown}} \times 30$ equals mg. nitrogen per 100 c.c. of blood where the standard is set at 20 mm.

Table I shows the comparative results obtained by the Folin-Wu and the selenium sulphuric acid methods. In Samples 13, 14, and 15, the Folin-Svedberg method and its selenium sulphuric acid modification were used. The figures given represent the average of two determinations.

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DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

TISSUE: Preparation of Neurologic Material for Histologic Study, Kernohan, J. W. Am. J. Clin. Path. **4**: 410, 1934.

Method 1.—Fat staining method:

The methods following have been found very satisfactory.

1. Fix tissue in formol solution, freeze and section, 10 to 20 microns.
2. Wash sections in 70 per cent alcohol for one minute.
3. Stain for five minutes in fat stain, made as follows:

Sharlach R, 1 gm. (Sudan IV may also be used)
Absolute alcohol, 70 c.c.
Distilled water, 30 c.c.

(Heat this mixture just a little, and shake while heating. Then add 100 c.c. acetone and put in a 37° C. oven overnight. Always filter stain before using.)

4. Wash in 70 per cent alcohol quickly.
5. Stain in hematoxylin (Harris) for five minutes.
6. Wash in tap water.
7. Dip in (1 per cent) acid (70 per cent) alcohol about two seconds.
8. Wash in tap water ten minutes or longer.
9. Wash in lithium carbonate (saturated aqueous solution) about two seconds.
10. Wash in tap water and mount with glycerin.

Method 2.—Perdrau impregnation method:

1. Paraffin sections run down to water in usual way.
2. Place for twenty to thirty minutes in 0.25 per cent solution of potassium permanganate.
3. Wash in water.
4. Place in solution of equal parts of 1 per cent oxalic acid and 1 per cent potassium sulphite, or 5 per cent oxalic acid, until color is gone.
5. Wash for a half to one hour in tap water until all acid is washed out (limit the length of time in case the sections come loose).
6. Place sections in a 20 per cent solution of silver nitrate for half an hour.
7. Wash quickly in double-distilled water.
8. Place sections in the following solution:

Silver nitrate (20 per cent) solution, 5 c.c.
Sodium hydroxide, 6 drops.

(Add ammonium hydrate drop by drop until the precipitate is almost destroyed. Add distilled water up to 50 c.c. and filter. Leave sections in this solution for half an hour.)

9. Wash in double-distilled water.
10. Fix in 10 per cent formol solution (neutral).
11. Wash in double-distilled water.
12. Tone in gold chloride solution 1:500.
13. Fix in sodium hyposulphite.
14. Wash in tap water, dehydrate, clear, and mount.

Method 3.—Toluidine blue, or thionin, staining method:

1. Run sections down to water.
2. Place sections in toluidine blue or thionin, stain for fifteen to twenty minutes (heat stain before using for thirty minutes): 1:500 solution = 1 gm. toluidine blue or thionin to 500 c.c. water.

3. Wash in two changes of 95 per cent alcohol (color comes out rapidly).

4. Place in absolute alcohol and watch under microscope until differentiation is complete.

5. Clear in xylol, thoroughly, and mount with Canada balsam.

Method 4.—Modified Bielschowsky impregnation method:

1. Fix tissue in solution of formol, 10 per cent.
2. Embed section in paraffin and cut at 10 microns.
3. Deparaffinize.
4. Wash three times in double-distilled water.
5. Place in 20 per cent solution of silver nitrate for one hour.
6. Wash quickly (twice) in double-distilled water.
7. Transfer sections into ammoniated solution of silver nitrate for five minutes: To 40 c.c. of 20 per cent silver nitrate solution add ammonium hydrate drop by drop until precipitate is dissolved. An excess of ammonium hydrate is noxious. Make a fresh solution each time it is to be used. Filter before using.
8. Wash quickly in double-distilled water.
9. Transfer sections into 10 per cent solution of formol (neutral) for one minute.
10. Wash, tone in gold chloride (brown) solution (a), fix in sodium hyposulphite solution (b) one or two minutes, clear (c), and mount (d):

(a) Gold chloride, 1 gm.
Water, 500 c.c. (can be kept as stock solution).

(b) Sodium hyposulphite, 5 gm.
Water, 100 c.c.

(c) 95 per cent alcohol.
Acetone.
Carbo-xylol.
Xylol.

(d) With Canada balsam.

Method 5.—Mallory-Heidenhain's staining method:

1. As a fixative, use formol or Zenker's solution, or Weigert's mordants. Cut paraffin sections at 5 microns.
2. Stain forty minutes in:

Azo-carmine (Grubler's), 1 gm.
Water, 100 c.c.
(Heat, cool, and filter at room temperature and add 1 c.c. of glacial acetic acid before using.)

3. Wash in water.
4. Differentiate in aniline alcohol (watch under microscope) until nuclei are red and cytoplasm is pale pink. This step requires from three to ten minutes depending on thickness of sections.
5. Remove aniline alcohol with acid alcohol (1 per cent) a half to one minute.
6. Wash quickly in water.
7. Place in 5 per cent phosphotungstic acid for three hours.
8. Wash quickly in water.
9. Stain one-fourth to one-half hour in Mallory's aniline blue made as follows:

Aniline blue, 0.5 gm.
Orange G, 2 gm.
Water, 100 c.c.
Acetic acid, 8 c.c.
(Boil, cool, filter, and thin with equal parts of water before using.)

10. Wash quickly in water.
11. Differentiate in absolute alcohol.
12. Clear in carbo-xylol and xylol.
13. Mount with Canada balsam.

Method 6.—Mallory's phosphotungstic acid-hematoxylin staining method:

1. Fix in Zenker's solution for twenty-four hours.
2. Place in running water for twenty-four hours.
3. Embed in paraffin.
4. Section and deparaffinize.
5. Place in potassium permanganate for from five to twenty minutes (make a fresh 0.25 per cent aqueous solution each time).
6. Wash well in water.
7. Place in oxalic acid (5 per cent aqueous solution) for from five to ten minutes.
8. Wash thoroughly in several changes of water.
9. Stain with Mallory's phosphotungstic acid-hematoxylin stain three to twelve hours. This stain is made as follows:

Ammonia hematin, 0.1 gm.
Water, 100 c.c.
Phosphotungstic acid crystals (Merck), 2 gm.

(Dissolve the ammonia hematin in a little water, with the aid of heat, and add it when cool to the rest of the solution; no preservative is required. If the solution stains weakly at first, it may be ripened by the addition of 5 c.c. of a 0.25 per cent aqueous solution of potassium permanganate, or it may be allowed to stand for a few weeks until it ripens spontaneously. Hematoxylin may be used instead of ammonia hematin, but requires 10 c.c. of the permanganate solution to ripen it.)

10. Place sections in 95 per cent alcohol, then in absolute alcohol, and clear in xylol.
11. Mount with Canada balsam.

Method 7.—Weigert's mordant I:

Potassium bichromate, 5 gm.
Fluorochrome, 2 gm.
Water, 100 c.c.
(Fix tissue in mordant I for from four to five days, for small and thin sections, two days.)

Method 8.—Weigert's mordant II:

Acetate of copper, 5 gm.
Fluorochrome, 2.5 gm.
Acetic acid (36 per cent), 5 c.c.
Water, 100 c.c.
Formol, 10 c.c.
(Fix tissue in mordant II for from twenty-four to forty-eight hours.)

Method 9.—Weigert's myelin-sheath staining method:

1. Wash tissue, which has been fixed in 10 per cent solution of formol, in water for short time.
2. Fix in primary mordant four to five days (for small and thin sections, two days).
3. Wash in water one hour.
4. Fix in secondary mordant twenty-four to forty-eight hours.
5. Wash well in water one to two hours.
6. Embed sections and deparaffinize.
7. Stain two hours in a solution made as follows:

10 c.c. of a 10 per cent solution of hematoxylin dissolved in absolute alcohol.
Saturated solution of lithium carbonate, few drops.
Distilled water, 90 c.c.
(Make this solution fresh each time.)

8. Wash in tap water.
9. To differentiate, place in a solution of:

Borax, 2 gm.
Potassium ferricyanide, 2.5 gm.
Water, 100 c.c.

10. Wash thoroughly in running water.
11. Dehydrate in 80 per cent and in 96 per cent alcohol.
12. Clear in acetone, carbo-xylol, and xylol.
13. Mount with Canada balsam.

(A stock solution of 10 per cent solution of hematoxylin in absolute alcohol should be kept on hand. At least ten days' exposure to sunlight is required to ripen this solution. Combine it with a few drops of saturated aqueous solution of lithium carbonate at the time of using.)

Method 10.—Cajal's gold chloride and sublimate method:

1. Place small portions of fresh tissue in the following solution for two to ten days (the tissue may have previously been fixed in formal solution a very short time):

Formol, neutral, 15 c.c.
Ammonium bromide, 1.5 to 2 gm.
Distilled water, 85 c.c.
(Tissue should not remain more than ten days in this solution, seven days is best.)

2. Cut sections on freezing microtome (20 to 25 microns) and place in 6 per cent neutral formol solution.
3. Wash a few moments in distilled water.
4. Place in following solution for six to ten hours and keep in the dark:

Gold chloride (1 per cent), 10 c.c.
Mercuric chloride, 0.5 gm. (10 c.c. of a 5 per cent solution).
Distilled water, 60 c.c.
(Dissolve the mercuric chloride by heat in nearly all water called for in the formula, filter, and add to the gold chloride. The best temperature is 18 to 20° C. At this temperature, four to six hours is the best. If temperature is 14 to 17° C., the time should be much longer. If temperature is higher, the time should only be two to three hours.)

5. After four or more hours, when sections are intense purple, fix for from six to ten minutes in the following solution:

Sodium hyposulphite, 5 c.c.
Alcohol (95 per cent), 30 c.c.
Distilled water, 70 c.c.
Saturated solution bisulphite of soda, 5 c.c.

6. Wash in 50 per cent alcohol.
7. Place on slide, blot carefully, then dehydrate on slide with 70 per cent, and 95 per cent, and absolute alcohol; then clear in xylol.
8. Mount in Canada balsam.

(Blot carefully after each of above steps. Oil of origanum may be used in place of absolute alcohol.)

Method 11.—Hortega silver carbonate impregnation method for oligodendroglia and microglia:

1. Fix tissue in formol-ammonium bromide solution (same as for Cajal's stain) and cut frozen sections.
2. Wash in two dishes of distilled water, the first containing 10 drops of ammonia.
3. Stain in undiluted silver carbonate for from one minute to one and a half hours. Del Rio Hortega's undiluted ammoniacal silver carbonate is prepared as follows:

Solution of silver nitrate (Merck) 10 per cent, 5 c.c.
Solution of sodium carbonate (pure) 5 per cent, 20 c.c.
Ammonius hydroxide, sufficient to dissolve precipitate.
(The ammonium hydroxide should be added drop by drop until the precipitate is just dissolved, stirring the solution all the while.) Finally, filter the solution and place in a dark bottle, where it will keep for long periods.

4. Wash rapidly in 60 per cent alcohol (made by using absolute alcohol). The sections should be carried through with a small angulated glass rod so as to allow all of it to be washed equally without wasting time. If the section is wrinkled or folded, the alcohol will produce a patchy result.

5. Reduce by passing sections directly in 1 per cent formol solution.

6. Wash in distilled water.

7. Tone by placing sections in a gold chloride bath ten to fifteen minutes until they become purple gray.

Gold chloride (yellow), 1 gm.
Distilled water, 500 c.c.

8. Fix in a 5 per cent solution of sodium hyposulphite for half a minute or more until sections are flexible.

9. Wash in water.

10. Dehydrate in dishes of graded alcohol and clear in carbo-xylol and xylol.

11. Mount with Canada balsam.

LIVER FUNCTION, In Pregnancy, The Bilirubin Excretion Test In, Sullivan, C. F., Tew, W. P., and Watson, E. M. Brit. J. Obst. & Gynec. 41: 347, 1934.

The investigation of liver function by means of the bilirubin excretion test of von Bergmann in pregnancy and its complications, as described in this paper, permits the following inferences:

1. During the first half of normal pregnancy the liver function, as determined by the bilirubin excretion test, is unimpaired, but during the second half of normal pregnancy, evidence of disturbed function can be demonstrated in at least 30 per cent of cases.

2. The cause of the impaired excretory power of the liver during the later stages of normal pregnancy is undetermined, but the fact of its existence renders the interpretation of hepatic efficiency tests in abnormal cases difficult.

3. Toxic patients, with signs of renal insufficiency, tend to show less retention of injected bilirubin than those with normal kidney functions. Thus, to some extent at least, it is possible to differentiate the toxemias of pregnancy into nephritic and hepatic types. So far as the bilirubin excretion test is concerned, there is not sufficient difference between the findings in toxic patients and the results obtained in normal women during the later months of pregnancy to be of practical significance. In certain instances, however, especially during the early stages of pregnancy or in cases of extreme toxemia, the test may supply information of clinical importance.

4. That the impairment of liver function which occurs during pregnancy, both normal and abnormal, is of a temporary nature is indicated by the tendency for the results of the bilirubin excretion test to return to normal following termination of the pregnancy.

5. Owing to its limited usefulness and the technical difficulties involved, the bilirubin excretion test is unlikely to become popular as a routine procedure in the practice of obstetrics.

The test procedure is as follows:

An amount of bilirubin equivalent to 1 mg. per kilogram of body weight is dissolved in 15 c.c. of 0.1 M. solution of sodium carbonate, previously boiled and cooled to about 80° C. After the pigment has dissolved completely the solution, having further cooled nearly to body temperature, is transferred to a 20 c.c. syringe and injected slowly into a vein at the elbow. Untoward effects of any kind have not been observed to follow such injection. Blood samples are obtained immediately before the injection and four minutes, thirty minutes, two hours, and four hours afterward. These samples, which are received in centrifuge tubes containing potassium oxalate, are centrifuged at once and kept in a refrigerator until the final collection has been made.

The concentration of bilirubin in each sample of blood plasma is estimated by the method of Ernst and Foster which procedure consists essentially of the simultaneous precipitation of the plasma proteins and the extraction of the bilirubin with acetone. For this

purpose 2 c.c. of acetone are added to 1 c.c. of plasma in a centrifuge tube. After shaking the plasma and acetone mixture, the precipitate is removed by centrifugalization and the supernatant liquid which contains the pigment is filtered directly into a microcolorimeter cup through a No. 40 Watman filter paper and compared with a standard composed of 1 in 6,000 potassium dichromate. It was found that clearer filtrates could be obtained if the centrifuge tubes, following the removal of the protein, were stoppered and chilled by immersion in a water-bath containing lumps of ice for five minutes before filtration. The acetone solutions must be protected from the light previous to making the color comparisons.

It is true that this method of estimation includes not only the bilirubin but other pigments such as carotin and lipochromes should these be present in the plasma, but in the cases reported in this paper, any effect produced by such substances was minimized by carrying out the tests with the patients in the fasting state. Moreover, assuming any error due to the presence of these nonbiliary pigments to be constant throughout the period of the test, their influence is removed completely by subtracting the result obtained in the preliminary control sample from the values found in the subsequent ones. Hence the bilirubin content of the specimen secured four minutes after the injection of the bilirubin minus that of the control sample is regarded as representing the maximum increment caused by the added pigment and is consequently recorded as 100 per cent. With this value as a basis, the findings for the other samples are expressed accordingly. While the results so obtained represent the relative rather than the absolute bilirubin concentrations, they do provide a representation of the completeness of elimination of the injected bilirubin in a specified time.

BLOOD SEDIMENTATION, Meteorologic Effects On, Hoverson, E. T., and Petersen, W. F. Am. J. M. Sc. **188:** 455, 1934.

There occur wide daily variations in the erythrocyte sedimentation rate, and at times the daily variation is as much as 100 per cent.

In general paresis, the rate of settling is slower than in "normal" individuals.

There is a correlation between the daily variations of the sedimentation time and the meteorologic changes.

It is believed that these meteorologic changes account for daily variations in the rate of settling.

PERIARTERITIS NODOSA, Associated With Rheumatic Heart Disease, Friedberg, C. K., and Gross, L. Arch. Int. Med. **54:** 172, 1934.

Four cases that came to autopsy are presented, in which widespread periarteritis nodosa was associated with rheumatic fever and rheumatic heart disease; the latter was confirmed by the presence of Aschoff bodies in the myocardium.

These four were discovered in a series of eight cases of periarteritis nodosa which came to autopsy in the course of two years. Prior to this period there were five additional cases which came to autopsy. Two of the five patients had a rheumatic history and evidence of rheumatic valvular disease. Verrucous endocarditis was disclosed in both cases at postmortem examination.

Criteria for the diagnosis of rheumatic infection and of periarteritis nodosa are discussed. On the basis of these criteria, none of the cases of periarteritis reported in the literature presented adequate evidence of rheumatic heart disease. Conversely, none of the vascular lesions described in rheumatic fever could be truly called periarteritis nodosa.

Because of the frequency of the association of these diseases in our cases and the simultaneous occurrence of the symptoms of each, we believe it probable that rheumatic fever is a common cause of the vascular lesions termed periarteritis nodosa.

In two of the cases an attack of scarlet fever occurred eight weeks before the symptoms of the other ailments became manifest. This point is briefly discussed.

In another case there was clinical and pathologic evidence of malignant sclerosis. This is mentioned in connection with Fahr's belief that rheumatic fever is one of the causes of malignant sclerosis.

In two of the cases the abdominal symptoms, so common in periarteritis nodosa, dominated the clinical picture sufficiently to lead to an exploratory operation. The authors suggest that when acute abdominal symptoms are present in a patient suffering from rheumatic fever, complicating periarteritis nodosa should be considered. This complication is offered as an organic basis for some of the instances of so-called abdominal rheumatism.

HOOKWORM, Comparison of Efficiency of Stoll Egg-Counting Technic With Simple Smear Method in Diagnosis of, Keller, A. E. Am. J. Hyg. **20:** 307, 1934.

In order to compare the efficiency of the Stoll egg-counting technic with the simple smear method in the diagnosis of hookworm, 2,412 specimens of feces were examined according to the following plan: Each specimen was examined one time by the dilution egg-counting technic and the simple smear method. The specimens which were positive by egg count but negative by smear or positive by smear and negative by egg count were reexamined by the method which failed to reveal hookworm eggs on the first examination.

On one examination 42.2 per cent of the specimens were positive by the egg-counting technic and 35.1 per cent were positive by the smear method. Forty-five additional positive specimens were found by the egg-counting examination which increased the total positive by egg count to 44 per cent. One hundred four additional positive specimens were found by the smear examination, increasing the total positives to 39.4 per cent.

In this series of examinations 7.1 per cent more specimens were found positive by one egg count than by one smear examination and 4.6 per cent after reexamination of the negatives which were not diagnosed by the first smear examination.

The greatest discrepancy between the two methods occurred in the lowest intensity groups where from 57.8 to 68 per cent of the positive specimens by egg count were positive by the smear method. When the intensity of infestation reached 2,000 eggs per gram of feces 95.1 per cent of all the specimens found positive by egg count were positive by smear. All of the specimens in the intensity group containing 5,000 eggs or more per gram of feces were positive by smear.

It was found for this series that the lowest level of intensity of infestation at which the smear, for practical purposes, would be of value in the diagnosis of hookworm infestation would be 1,200 eggs per gram of feces rather than 500 eggs per gram as has been determined by other investigators.

The positive specimens missed by two egg counts were as a rule light infestations (50 worms or less) but an occasional high count would be missed by this technic.

A correlation of the relative number of eggs per smear with the intensity of infestation as found by the egg-counting technic was made of 931 specimens. In the smear group containing from one to five hookworm eggs per slide there was an average count of 1,690 eggs per gram (approximately 65 worms). In the smear group containing from 6 to 25 eggs per slide the average intensity of infestation was 4,190 eggs per gram (approximately 160 worms) and in the group containing from 26 to 40 eggs per slide by the smear method the average count was 12,325 eggs per gram (approximately 492 worms). When there were 41 or more eggs per slide by smear examination the average egg count was 23,100 eggs per gram (approximately 924 worms).

These results suggest that it may be possible to correlate the number of hookworm eggs found per smear with the intensity of infestation as found by the dilution egg count examination.

These data show that the dilution egg-counting technic is more accurate than the smear method for this series of examinations and that the results of investigations which have been conducted in which the Stoll egg-counting technic has been employed are dependable when compared with the results obtained by the examination of specimens by the smear method.

PNEUMOCONIOSIS, Pathology of: Review, T.H.B. Am. J. M. Sc. **188:** 418, 1934.

From the data assembled and discussed in this rather comprehensive review the following conclusions are drawn:

There is no conclusive evidence to show that silica has a specific action on the tissues.

Certain of the silicates and perhaps other substances may produce similar if not identical effects.

Infection, and especially tuberculous infection, is a more serious factor in the development of the disabling pneumoconioses than dust per se.

Dust modifies pulmonary tuberculosis in a twofold manner; it increases the fibrous response, thus promoting chronicity, and at the same time it increases the tendency of the infection to spread.

The so-called "silicotic nodule" has probably never been reproduced experimentally except by the synchronous inoculation of tubercle bacilli with dust.

The "silicotic nodule" is probably always a tuberculous lesion, modified by dust.

Chemical assay of suspected tissues is open to the criticism that it does not reveal the proportion of silica to silicates.

There is probably no such thing as acute silicosis.

Evidence regarding the action of coal dust on the tissues and its influence on tuberculous infection is conflicting.

TRICHINOSIS, Anomalous and Nonspecific Reactions With T. Spiralis Antigen in Relation to Other Disease Conditions, Bachman, G. W., Molina, R. R., and Gonzalez, J. O. Am. J. Hyg. **20:** 415, 1934.

The fact that human trichiniasis has not been found in Porto Rica enabled the authors to study the specificity of the precipitation test in relation to other disease conditions existing in the Island.

Anomalous and nonspecific precipitation reactions were studied in serums from 857 apparently trichinella-free individuals living in Porto Rica. These individuals were classified into 6 groups: A, B, C, D, E and F, with relation to the various disease conditions affecting them at the time the test was made.

From these 6 groups 857 serums were tested, of which 35 (or 4.8 per cent) gave an anomalous type of reaction, 72 (or 8.4 per cent) gave nonspecific precipitation reactions, and 750 (or 87.5 per cent) gave negative reactions.

Group A: In 98 individuals who gave a positive Wassermann and Kahn reaction, 2.0 per cent gave anomalous types of reaction, and 8.1 per cent gave nonspecific precipitation reactions. In 573 individuals, negative for Wassermann and Kahn, 0.34 per cent gave anomalous reactions, 4.7 per cent gave nonspecific reactions, and 94.9 per cent were negative.

Group B: In 39 individuals of 81 malaria cases (positive laboratory diagnosis), all of whom had received quinine, 7.69 per cent gave anomalous reactions, 12.9 per cent, nonspecific reactions, and 79.48 per cent, negative reactions. Of the remaining 42 individuals who did not take quinine, 9.52 per cent gave anomalous reactions, 26.19 per cent gave nonspecific reactions and 64.28 per cent gave negative reactions. The results obtained in this group seem to indicate that a positive or negative history of quininization previous to the precipitation tests had no effect on the number of anomalous reactions.

Group C: In 42 individuals clinically diagnosed as malarial cases, 2.38 per cent gave anomalous reactions, 2.38 per cent, nonspecific, and 95.2 per cent, negative. In this group the lowest percentage (2.38 per cent) of nonspecific reactions was found for all the groups.

Group D: In serums from 169 individuals harboring one or more intestinal nematodes, only one case that harbored Necator americanus gave an anomalous reaction and one nonspecific reaction. None of the cases harboring Ascaris or trichuris gave either anomalous or nonspecific precipitation reaction.

Group E: In the serums from 154 hospitalized cases suffering from one or more disease conditions, there were no anomalous reactions. Fourteen (9.09 per cent) gave nonspecific reactions, and 140 (90.9 per cent), negative.

Group F: In the serums from 7 individuals who showed nitrogen retention, increased cholesterol and chlorides in their blood, two anomalous reactions occurred. In this small group 71.4 per cent gave nonspecific precipitation reactions, the highest observed in all of the 6 groups.

Of the 857 serums studied from the above groups, the titers varied from 1:100 to 1:3,000. In the precipitation test of the various groups, 18.06 per cent gave a titer of 1:100; 19.4 per cent, 1:200; 41.6 per cent, 1:500; 11.9 per cent, 1:1,000; 4.0 per cent, 1:1,500; 2.8 per cent, 1:2,000; 2.08 per cent, 1:3,000.

We may deduce from these investigations that the precipitation test for the diagnosis of human trichiniasis does possess a fairly high specificity in relation to other disease conditions. It is the experience of the authors that the presence of the anomalous reactions can be easily differentiated from the true, positive precipitation rings. According to the results of the authors, nonspecific precipitation reactions occur in low dilutions at the interphase of the serum and test antigen, and give rings similar to a true precipitation ring in Wassermann and Kahn reactions and parasitic infestations (Group E) as well as in conditions where there is nitrogen retention and increased cholesterol and chlorides in the blood.

A titer of 2,500 and above in terms of dry weight of powder can, according to the experience of the authors, be termed specific for trichiniasis in 90 per cent of cases.

Erratum

In the article by William P. Belk in the July issue of THE JOURNAL OF LABORATORY AND CLINICAL MEDICINE, page 1035, the author's name and title should read:

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The following bibliography is added:

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EDITORIAL

Vaccination Against Acute Anterior Poliomyelitis

THAT monkeys can be successfully vaccinated against acute anterior poliomyelitis by subcutaneous and intracutaneous injections of the virus has been amply demonstrated,[1] but the method has been considered too risky for the immunization of human beings.

Kolmer and Rule,[2] however, have found that monkeys can be safely and very effectively vaccinated with virus slightly attenuated by sodium ricinoleate. Subcutaneous injections of amounts as high as 0.05 to 1 c.c. per kg. of a vaccine of 4 per cent suspensions of monkey spinal cord virus in 1 per cent solutions of this agent for as many as ten consecutive injections, have produced no evidence of infection while immunizing the animals to intracerebral injections of ten or more infective doses of highly virulent virus. Furthermore, the immunity has been found to endure for at least three years.

Following the experiments these investigators took three subcutaneous injections of the vaccine with no ill effects and subsequently administered it to a group of twenty-five children varying in age from eight months to fifteen years by subcutaneous injection at weekly intervals for 2 to 3 doses.[3] Only slight reactions occurred at the sites of inoculation with no ill effects and with the production of antiviral antibody in the blood of about 85 per cent believed to be sufficient for affording protection against the disease.

Since then over 5,000 children have been immunized in different parts of the country[4] but especially in North Carolina, Virginia, Tennessee and neighboring states where an epidemic began in June. Only slight local reactions have occurred at the sites of injection although a very careful aseptic technic must be observed in its administration because of the low bacteriostatic and bactericidal effects of sodium ricinoleate. An occasional severe local reaction has been found due to accidental bacterial contamination of the vaccine, although 1:60,000 phenyl mercuric nitrate is now being added which has been found sufficient for bacteriostatic effects and without appreciable reduction in the immunizing properties of the vaccine.[4]

It would appear therefore that this vaccine of living attenuated virus has been proved safe for the immunization of human beings. Furthermore, the brain and spinal cord of every monkey removed at autopsy are being tested for the possible presence of the virus of lymphocytic choriomeningitis of Armstrong and Lillie[5] by intracerebral injection into mice and guinea pigs before the cord is used in the preparation of the vaccine, but so far tests with a large number of monkeys have not shown the presence of this virus.[4] It is believed by Kolmer and his associates that the safety of the vaccine may depend upon the possibility and probability that the remote monkey passage virus employed in its preparation has lost infectivity for human beings. Furthermore, the subcutaneous route of administration, a small first dose, and the rapidity of antibody production are believed to be additional factors of safety. While attenuation of the virus by sodium ricinoleate may be an additional factor of safety, yet the degree of attenuation is so slight as to be a factor of much lesser importance.

Whether or not the effectiveness of the vaccine in the prevention of infantile paralysis will be as definitely shown as has its safety remains to be determined. But the fact that it has proved highly effective in the protection of monkeys and that the antibody it has engendered has neutralized human virus from the California epidemic[3] appears to indicate that it will prove highly effective. While Kolmer and his associates have found antiviral antibody in the blood of some children as early as four days after the first injection,[3] it may be that it will fail to abort the disease when given late in the incubation period.

Vaccines of virus killed by heat and various chemical agents have proved much less effective in the immunization of monkeys. Park and Brodie of New York, however, have found that large doses of a 20 per cent suspension of monkey spinal cord virus believed to be "killed" with 0.1 per cent formalin were effective in the immunization of monkeys and that two doses by sub-

cutaneous and intracutaneous injection produced antibody lasting for about five months in the blood of about 85 per cent of children. This vaccine has been used to some extent in the California epidemic and is also being tried in the North Carolina epidemic at the present time. As stated by Andrewes,[6] however, it would appear definitely established that vaccines of dead viruses are enormously weaker than living and attenuated viruses because the former contain but small amounts of antigenic virus protein, while the virus in the latter after injection can multiply, perhaps, a million fold in the body and thereby have a far better chance of producing resistance.

If, therefore, the Kolmer vaccine is proved completely safe, as appears to be the case, it may prove to be the more effective and capable of engendering a greater degree of immunity of longer duration. At any rate both vaccines are now being given a thorough trial in the North Carolina epidemic and the results will be awaited with a great deal of interest. And, while the attack rate of the disease is normally low, yet this is so much increased during epidemics that it is greatly hoped that one or both of these vaccines will prove effective in protecting children over the age of greatest susceptibility until the natural immunity occurring in about 80 per cent of adults has been acquired.

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—*W. T. V.*